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Intra-Pituitary Administration Revisited: Development of a Novel *in Vivo* Approach to Investigate the Ovine Hypophysis

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Abstract

The anterior pituitary gland regulates physiological processes via the secretion of hormones, which are under the control of factors produced either in the hypothalamus or the pituitary gland itself. Studies investigating how the pituitary gland functions have employed both *in vitro* and *in vivo* approaches. Although *in vitro* analysis has the advantage that it is pituitary specific, the results may be incomplete because the tissue is isolated from other physiological inputs that could affect function under natural conditions. Without vascular input, such studies are inherently of short duration. Conversely, *in vivo* experiments that rely upon systemic hormone injections require high doses, are non-target specific and the precise hormone concentrations reaching the pituitary gland are difficult to control. Intracerebroventricular hormone infusions are reliant on assumptions that factors are transported to the pituitary gland from the cerebrospinal fluid and are without cerebral effects. Here we describe an innovative method to investigate anterior pituitary function in conscious sheep by direct infusion of peptides into the pituitary tissue surrounding the hypophyseal portal blood vessels. This approach is an adaptation of the hypophyseal portal cannulation technique whereby an indwelling cannula provides direct access to the rostral aspect of the adenohypophysis. Peptide infusions were achieved by insertion of a needle through the implanted cannula such that it penetrated the pituitary. Using this technique, infusion of TRH (17ng/1μl/min for up to 6h) induced a sustained rise in systemic prolactin levels that lasted for the duration of the infusion.

Keywords

pituitary; *in vivo*; chronic; neuroendocrinology

1. Introduction

Understanding how the pituitary functions has been central to the field of endocrinology since its inception (Kaplan, 2007). The anterior pituitary gland synthesizes and secretes at least seven established hormones, with the release of these being controlled primarily by factors produced either in the hypothalamus or the pituitary gland itself. Hypothalamic factors are released into the primary hypophyseal portal plexus in the median eminence,

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from where they are transported into the pars distalis of the anterior pituitary gland. Pituitary factors access their target sites by either diffusion, to act via a paracrine or autocrine pathway (e.g. prolactin effects on LH (Hodson et al., 2010)) and/or are released by the pars tuberalis into the portal vessels (e.g. “tuberalin” regulation of prolactin (Dupre et al., 2010)) in a manner similar to regulatory hypothalamic factors.

Elucidation of the mechanisms involved in regulation of anterior pituitary hormone secretion has involved both *in vitro* and *in vivo* techniques. *In vitro* approaches have the advantage that they are tissue specific, for example pars distalis tissue can be isolated and exposed to potential releasing hormones under controlled experimental conditions. This isolation comes at a cost, however, because the tissue is not being investigated under natural conditions, where other physiological inputs (such as from the brain, or the systemic circulation) may affect the outcome. Furthermore, isolated tissue has no vascular input, so such studies are inherently of short duration and the investigation of chronic regulatory effects, such as seasonal changes in hormone release are not possible. *In vivo* experiments allow cell functions to be investigated under natural physiological conditions, where normal external inputs are present. Manipulation of the hormonal output of the pituitary gland by injection of factors into the systemic circulation is, however, problematic for a number of reasons. First, the technique is not tissue specific and there is the inherent limitation that the injected factors interact with non-target tissues expressing the relevant receptors. For example, corticotrophin-releasing hormone (CRH) receptors are found in multiple neural (Jaferi and Pickel, 2009) as well as non-neural (Chatzaki et al., 2004) loci. Similarly, follistatin is a hormone centrally implicated in the regulation of FSH, yet *in vivo* studies on this hormone are offset by evidence that this peptide has significant effects on muscle (Lee et al., 2010). Secondly, many peptide hormones are quickly broken down by enzymes in the peripheral circulation, so large doses need to be injected to ensure that sufficient concentrations reach the intended target cells. Not only is this expensive, but injecting a large dose of a peptide into the general circulation may have undesirable pharmacological effects on homeostasis. For example, significant amounts of follistatin, which is hypothesized to work through an intra-pituitary paracrine pathway (Deneff, 2008), are required for *in vivo* effects, especially in large animal models (Padmanabhan et al., 2002). High doses of factors administered intravenously may also cross the blood-brain barrier, with possible cerebral effects (Caraty and Skinner, 2008). Third, it is extremely difficult to regulate, or even estimate, the concentration that eventually reaches the site of interest or the duration of exposure to the factor under investigation.

A second *in vivo* technique used to manipulate hormonal secretion from the pituitary is intra-cerebroventricular (icv) infusion of hormones and peptides. This method allows factors to be placed close to the median eminence, but again result in non-target cell exposure with its potential effects, although this may be more limited than with systemic treatments. This approach also depends on the hypothesis that infused factors are transported from the CSF to the pituitary gland, possibly via tanycytes (Rodriguez et al., 2005). However, we have provided compelling evidence that this is not always the case, as icv infused GnRH did not enter the hypophyseal portal circulation (Skinner et al., 1998). Finally, it is noteworthy that investigations in which substances are administered icv may produce results at odds (Caraty et al., 1997) with studies that use the intravenous route (Donald et al., 1983), which, again, may indicate the presence of sites of action beyond the intended target.

Some of the earliest studies in the field of neuroendocrinology used a technique of direct pituitary injection, with cannulae implanted through the top of the cranium (Campbell et al., 1964; Domanski and Kochman, 1968). However, this approach resulted in some damage to the hypothalamus and fell out of favor; we are unaware of studies using this technique to investigate the specific release of hormones from the pituitary gland. While this technique

was used mainly in small mammals (Domanski and Kielanowski, 1966), it lends itself to investigations using larger animal models, such as sheep. Here we report a novel adaptation of the hypophyseal portal sampling technique (Caraty et al., 1988; Clarke and Cummins, 1982) to allow for the direct infusion of peptides into the anterior pituitary in fully-conscious sheep. This technique permits the pituitary gland to operate in its normal physiological milieu, where it is not isolated from other physiological systems. Moreover, it circumvents the systemic circulatory system, allowing greater control over the concentrations and flow rates of experimental factors to be administered. In addition, it allows prolonged infusions over many hours or even days and, if sterility is maintained, it can be repeated with different factors at different times within the same animal.

The essential elements of effective delivery of factors into the pituitary gland are: 1) the infusion needle must penetrate the tissue to allow factors to reach target cells (either by hypophyseal portal blood or diffusion); 2) the needle must stay in a fixed position for the duration of the treatment; 3) the rate of infusion must be very slow ($\sim 1\mu\text{l}/\text{min}$) to avoid physical damage to the tissue. To demonstrate the functionality of the intra-pituitary approach we developed, we infused thyrotropin-releasing hormone (TRH), which is an established and potent stimulator of prolactin release (Samson et al., 1998), into the anterior pituitary gland and measured prolactin levels in systemic blood samples collected during and after the infusion. As prolactin is responsive to stress, this hormone was also selected as it would help to discriminate between *de facto* responses to TRH from responses to mechanical pituitary manipulations.

2. Materials and Methods

2.1 Animals

Adult ewes of mixed breed were kept outside ($41^{\circ} 10' \text{ N}$) until surgery, after which they were maintained inside under artificial light conditions corresponding to the prevailing photoperiod and provided with hay and water *ad libitum*. Twenty eight intact ewes were sampled in total: January ($n=6$), April ($n=11$), June ($n=5$), September ($n=3$) and December ($n=3$). Access to hay and water was restricted during infusion experiments to avoid potential postprandial changes (Trenkle, 1978) in prolactin secretion. At the completion of each experimental sampling period, ewes were euthanized with an overdose of sodium pentobarbital (Beuthanasia, Schering-Plough, Union, NJ) to check the precise placement of indwelling cannulae and determine the extent of penetration of the needle into the pituitary gland. The experimental procedure used in this study was approved by the University of Wyoming Animal Use and Care Committee.

2.2 The indwelling cannula

The indwelling cannula (Fig. 1A) was constructed from a single blunted 12 gauge hypodermic needle (length 110 – 115 mm) with a plastic cup (derived from the cone shaped end of a 0.5 ml micro-centrifuge tube) glued to the distal end, and was a modification of a two needle device described by Caraty *et al.* (1994). The total length of the device was between 115 and 125 mm with the precise length used with each animal dependent on the size of the sheep head.

2.3 Pituitary surgery and placement of cannula

The rostral face of the anterior pituitary, covered with the dura mater was exposed using the methods described (Caraty and Locatelli, 1988; Caraty et al., 1994). The distance from the end of the cup to the anterior face of the pituitary was estimated by pushing a blunt probe through the needle until it met resistance. The cannula was removed and the distance that the probe extended beyond the end of the cup measured. This distance, plus the length of the

cannula gave the estimated length of the infusion needle (Fig. 1B) needed to penetrate the pituitary. The dura mater was not cut during surgery to reduce the risk of infection before infusions. The cannula was placed so that the plastic cup rested against the bone remaining at the end of the tunnel. The cannula was fixed in place by backfilling the tunnel with dental acrylic (Fig. 1C) and the skin incision was sutured closed, leaving only the hub of the needle exposed. This was temporarily closed with a stainless steel plug (plus attached obturator, which filled the length of the cannula) to maintain sterile conditions.

2.4 Infusion needles

The infusion needle was constructed from two different needles glued together (Fig. 1B): a blunted 14G $\times$ 5.25" needle, taken from a Jorvet polyurethane extended use iv catheter (Jorgensen Labs, Loveland, CO); and a 22G $\times$ 1.5" hypodermic needle (Becton, Dickinson, Franklin Lakes, NJ) fixed inside the front tip of the 14G needle. The smaller 22G needle penetrates the pituitary to minimize physical damage to the tissue. To hold the infusion needle in place inside the indwelling cannula during treatments, the tip and shoulder of a 1ml regular luer syringe (slip tip) was attached to the rear of the 14G needle using adhesive bond. The distance from the shoulder of the syringe to the point of the 22G needle was fixed as the distance from the end of cannula to the dura mater (estimated during surgery) plus an additional 4mm, to ensure penetration past the zona tuberalis into the pars distalis.

2.5 Effect of needle placement, saline and TRH on prolactin

Ewes were allowed to recover post-surgery for one week before infusion studies commenced. A day before sampling, each ewe was fitted with an indwelling jugular catheter (Jorgensen Labs) and moved into an individual pen to acclimate. Pens were adjacent to each other and a minimum of three ewes were sampled at one time to avoid the stress of social isolation. Polyethylene extension tubes (Lectro-Cath, Vygon, Montgomeryville, PA) were attached to each jugular catheter and run back behind the ewe through a hole in the wall to a separate collection room to allow remote blood sampling. On sampling days, plasma was collected for 2h before infusion needles were inserted to obtain baseline prolactin levels. Infusion needles were connected to external pumps (Graseby Medical External Pump, Model MS26 syringe driver, MarCal Medical, Millersville, MD) using polyethylene extension tubing (L: 30cm; ID: 1mm; OD: 2mm; Vygon) which were fixed on the back of the neck of the ewe with tubular elastic dressing (Surgilast, Derma Sciences, Princeton, NJ). The infusion rate was 1 μ l/min.

The experimental protocols, with jugular blood samples collected every 20 minutes, are shown in Figure 2. As the technique involves some mechanical stimulation of the pituitary gland due to penetration of the hypodermic needle and infusion of small volumes, Experiment 1 infused both saline (0.9%; vehicle) and TRH (American Peptide, Sunnyvale, CA; 17ng/ μ l/min) for 2h on different days, with the order in which they were infused randomly varied. At the end of each infusion, the ewes remained in individual pens, where they were fed and watered until the next day, at which time infusions recommenced. In Experiment 2, to investigate the effect of duration of TRH infusion on prolactin release, we infused TRH for 4 or 6h (17ng/ μ l/min).

In a subset of the 2, 4 or 6h TRH infusions, we continued to collect blood samples for an additional 2h beyond the end of the infusion period. To minimize disturbances in this post-infusion period, the pumps were simply switched off.

All blood samples were collected into heparinized tubes (100 USP Units; APP Pharmaceuticals LLC, Schaumburg, IL) and stored at 4°C before being centrifuged (3200g; 20min; 4°C) within 90 min. Plasma was harvested and stored at -20°C.

2.6 Comparison of intra-pituitary TRH infusion with intravenous TRH infusion

In Experiment 3, we compared the effect of intra-pituitary with intravenous infusion on prolactin release. Two indwelling jugular catheters were inserted in each ewe ($n=3$), with one catheter used to collect blood every 20min, while the other was used to infuse TRH in saline vehicle. Saline infusions were used as controls. TRH was infused at a rate of 17ng/ μ l/min in both intra-pituitary and intravenous infusions.

2.7 Diffusion of infused factors within the pituitary gland

To determine the extent to which infused factors diffuse within the pituitary gland, 3% ammonium ferrous sulphate in 0.9% NaCl was infused (1 μ l/min for 1h; $n=2$). Immediately after infusion, ewes were euthanized with an overdose of sodium pentobarbital, decapitated, and their heads perfused through the carotid arteries with 1L 0.1% sodium nitrite plus 5% potassium ferrocyanide in 0.9% NaCl, and then 3L 4% paraformaldehyde in 0.01PBS. Pituitary glands were removed, stored for 24h in 4% paraformaldehyde, and transferred to 20% sucrose in 0.01PBS for storage. Sagittal pituitary sections (20 μ m) were cut on a cryostat, mounted on Silane-coated slides and stained with Cresyl violet. The volumes of the entire pituitaries as well as the volumes stained by ammonium ferrous sulphate infusions were estimated by taking digital images of sagittal sections of the pituitary (at 400 μ m intervals) while mounted on the cryostat. Cross-sectional areas of the pituitary gland and the stained areas were estimated by Image J and multiplying with section depth to estimate volume.

2.8 Radioimmunoassay

The concentration of prolactin in plasma samples was measured in duplicate by a double-antibody radioimmunoassay using ovine prolactin antigen (NIDDK-oPRL-1-3: National Hormone & Peptide Program) and ovine prolactin antiserum raised in rabbits (NIDDK-anti-oPRL-2). The RIA procedure was a modification of that described by McNeilly & Andrews (1974). Plasma and standards were incubated overnight (room temp) with assay buffer and antiserum, I¹²⁵-prolactin was then added and incubated for 24h before secondary goat anti-rabbit and a further 24h incubation. Antibody-bound and free hormones were separated by centrifugation (2500g; 30min; 4°C). All samples from an individual ewe were measured in the same assay. Assay sensitivity averaged 0.57 ± 0.21 ng/ml, and intra- and inter-assay coefficients of variation were 8% and 16% respectively (16 assays).

For three ewes, the concentration of LH in plasma samples was measured in duplicate by a double-antibody RIA using ovine LH antigen (NIDDK-oLH-1-4) and ovine LH antiserum raised in rabbits (NIDDK-anti-oLH-1). The RIA procedure was a modification of that described by Niswender *et al.* (1969). Plasma and standards were incubated overnight (room temp) with assay buffer and antiserum, I¹²⁵-LH was added and incubated (24h) and then secondary goat anti-rabbit was added for a further 24h. Antibody-bound hormone was separated from free by centrifugation (2500g; 30min; 4°C). All samples from an ewe were measured in a single assay. Assay sensitivity averaged 0.64 ± 0.18 ng/ml, and intra- and inter-assay coefficients of variation were 7% and 4% respectively (3 assays).

2.9 Data analysis

To investigate changes in concentrations of prolactin in the circulation resulting from needle insertion and infusions, prolactin concentrations for each ewe were standardized to give percentage change with time relative to the mean baseline before treatment. Prolactin concentrations of six consecutive samples, collected over 2h directly before treatment, were averaged, and this average was used as the baseline. All prolactin values before and after treatment were subsequently converted to percentage relative to baseline. Normalization was

performed due to seasonal variation in basal prolactin. The same normalization was performed on the LH data. To test for an effect of treatment on prolactin concentrations, monthly data were pooled and analyzed using Friedman's non-parametric test for repeated measures. This was necessary because Levene's test indicated that the data had unequal variance ($F_{\text{Levene}}=4.86$, $P<0.001$). Dunn's multiple comparison test was used to identify significant differences. To test for an effect of month on the prolactin response to TRH, the mean 2h prolactin concentration before and during TRH, the area under the curve (AUC) during the TRH infusion were calculated for each ewe, log transformed, and compared using one-way ANOVA. The relative increases in prolactin release (Post-TRH/Pre-TRH) were compared using one-way ANOVA. To investigate the effect of duration of TRH infusion on the prolactin response, percentage prolactin values from the 6h experiment were grouped into 2h segments (0–2h, 2–4h, and 4–6h post infusion). Data were analyzed using Friedman's non-parametric test for repeated measures, and Dunn's multiple comparison test to identify significant effects.

3. Results

In all ewes, the infusion needle penetrated the pituitary gland (Fig. 3). Vertical positioning of the needle along the face of the pituitary varied up to 5mm between ewes, with some needles in the dorsal pars distalis and some more medial with none in the ventral pars distalis. The needle penetrated ~6mm beyond the anterior wall of the pars distalis. The estimated volume of anterior pituitary affected by a 1h infusion of 3% ammonium ferrous sulphate was 71–83 mm³ (comprising ~7% of total pituitary volume).

As expected, pre-infusion prolactin concentrations were seasonal ($p<0.01$), and ranged from a peak in June (87.3 ± 9.4 ng/ml) to a nadir in January (18.3 ± 2.6 ng/ml), with intermediate concentrations evident in April (56.2 ± 12.0 ng/ml) and September (34.4 ± 2.4 ng/ml). Prolactin increased spontaneously after needle insertion in only 2 of 22 ewes and in only 2 of 24 ewes in response to saline (Fig. 4). The effects of needle insertion and saline infusion on the prolactin response were analyzed together ($n=19$), with ewes that responded to needle insertion or saline infusion excluded. There was no effect of time post-needle or -saline infusion on prolactin concentrations (Friedman statistic=21.21, $P=0.171$).

There was a robust and persistent increase in prolactin release in response to 2h, 4h and 6h TRH infusions (Fig. 5). TRH was infused in 21 ewes, four of which were excluded from the analyses because they responded to needle insertion or saline infusion. There was a significant increase in prolactin release in response to TRH in the remaining 17 ewes. For the 2h infusion data, there was a strong effect of time post infusion on prolactin levels (Friedman statistic=119.2, $P<0.001$) with Dunn's multiple comparison test indicating that prolactin increased significantly 20min after starting TRH infusion and remained significantly higher than baseline for the duration of the 2h infusion. For the washout period after TRH infusion terminated, a subset of the ewes were tested ($n=10$). There was a strong effect of time during and after TRH infusion on prolactin levels (Friedman statistic=125.0, $P<0.001$), with the multiple comparisons test indicating that prolactin was significantly elevated from baseline during TRH infusion and for a period of 40min after termination of infusion. One hour after termination of TRH infusion, prolactin levels declined and were not significantly different from baseline. During the 6h TRH infusion, prolactin secretion increased with time (Friedman statistic=8.0, $P=0.016$) and prolactin concentrations were significantly higher during the 4–6h period compared to the 0–2h period. There was a significant effect of season on mean prolactin release induced by TRH ($F=6.8$, $df=3$, $p=0.01$; Jan: 52 ± 18 ng/ml, Apr: 316 ± 65 ng/ml, Jun: 147 ± 28 ng/ml, Sept: 233 ± 108 ng/ml) as well as the AUC ($F=6.6$, $df=3$, $p<0.05$), but there was no significant difference in prolactin increase

relative to baseline ($F=1.8$, $df=3$, $p=0.2$; Jan: 5.1 ± 1.9 , Apr: 2.8 ± 0.6 , Jun: 2.2 ± 0.1 , Sept: 2.5 ± 0.3).

Intravenous infusion of TRH ($17\text{ng}/\mu\text{l}/\text{min}$) did not affect prolactin release, while intra-pituitary infusion of TRH at the same rate ($17\text{ng}/\mu\text{l}/\text{min}$) increased prolactin concentrations in the same ewes (Fig. 6).

Needle insertion, saline infusion and TRH infusion did not affect LH secretion (Fig. 7), including one ewe that showed a robust prolactin response to needle insertion.

4. Discussion

Our novel adaptation of the hypophyseal portal approach provides a new method of manipulating pituitary function by allowing infusion of peptides directly into the pars distalis. This obviates many problems associated with functional pituitary investigations. To validate the method, our results showed that needle insertion and saline infusion were without effect on prolactin secretion in the majority of animals. In the few ($<10\%$) cases where prolactin increased in response to either the needle or saline, there may have been a non-specific mechanical stimulation of lactotropes. Due to these rare technical artifacts, we recommend that the effect of needle insertion and vehicle infusion are determined for each animal and animals excluded from analysis accordingly. In contrast, infusion of TRH directly into the pituitary gland powerfully increased prolactin secretion as predicted in all but one animal. The method was shown to be repeatable within the same animals over several days as long as infection was prevented. This was achieved by sterilizing the infusion needles with 70% ethanol before insertion and by daily administration of long acting antibiotics.

As some pituitary hormones are sensitive to stress, and handling of sheep is a known stressor, this must be accounted for in the experimental design. Handling times can be minimized by good technique, sheep can be habituated to small amounts of handling before experiments commence and it is possible to allow a rest period after needle placement and prior to the start of any experimental treatment. To limit human exposure to the absolute minimum, blood sampling was performed remotely.

TRH is a known stimulator of prolactin in all species investigated, although the physiological function, if any, of TRH in the short-term regulation of prolactin release is unknown and may be pharmacological (Fraser and McNeilly, 1982; Shin, 1978; Thomas et al., 1988). However, it has been suggested that TRH may play a role in prolactin release during lactation (Yamada et al., 2006). In agreement with previous studies in the cow (Johke, 1978) and mare (Thompson et al., 1986), the amount of prolactin secreted in response to TRH was seasonally dependent in the ewe (lowest in Winter, highest in Spring/Summer), although the relative increase, compared to baseline, did not change with season. Although difficult to compare to other studies, it is noteworthy that the rate of intra-pituitary TRH infusion we used ($17\text{ng}/\mu\text{l}/\text{min}$) was ~ 30 fold lower than that used during iv infusion in sheep (Robinson et al., 1996; Taylor et al., 1986) and humans (Samuels et al., 1993). In the mare, a $5\mu\text{g}$ iv TRH injection was insufficient to stimulate prolactin secretion (Johnson, 1986). Importantly, we observed no increase in prolactin when this $17\text{ng}/\mu\text{l}/\text{min}$ rate of TRH was infused iv. Our finding that TRH-induced prolactin secretion is sustained is consistent with studies administering TRH systemically to goats (Hashizume et al., 2009) and sheep (Robinson et al., 1996). Taken with other studies (Hartnell et al., 1987; Robinson et al., 1996; Samuels et al., 1993), this suggests that the prolactin response to TRH may not desensitize, which is in contrast to some other neurohormone/G protein coupled receptor systems (e.g. the ACTH response to CRF (Evans et al., 1988)).

In summary, we have developed a novel method to infuse factors directly into the pituitary gland and measure the endocrine consequences *in vivo*. This approach will be particularly powerful when assessing the effect of factors, which when given iv, may affect multiple targets outside of the pituitary gland, rendering results equivocal at best. Indeed, this approach may resolve the difficulties highlighted recently when investigating elusive mechanisms controlling the seasonal secretion of prolactin (Dupre et al., 2010).

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Highlight

- Intra-pituitary infusion circumvents circulatory system
- Technique permits administration of factors that could affect peripheral targets if administered intravenously
- intra-pituitary TRH increase prolactin release
- prolactin release sustained and does not desensitize

Figure 1. Schematics of the A) indwelling cannula, B) infusion needle, which was constructed from a 22G needle attached to a blunted 14G needle with a luer slip tip appended to the rear, and C) position of cannula post surgery. This placement follows a similar approach to that of Caraty et al (1988), with the cannula apposed to the zona tuberalis region of the pituitary gland.

Figure 2.

Experimental design. In Experiment 1, the order in which saline and TRH were infused was alternated randomly. In Experiment 2, TRH was infused for 4 or 6h. The washout period, as indicated by the time between cessation of infusion and the end of sample collection, was not monitored in every ewe and is noted accordingly in the results. In Experiment 3, intra-pituitary (IP) TRH infusion was compared with intravenous (IV) TRH infusion.

Figure 3.

A) Dorsal view of needle penetration into the sella turcica after the pituitary has been removed, with the front of the head on the left. B) Anterior face of the pituitary gland noting the penetration points of two needles (arrow heads). C) Sagittally cut tissue block on cryostat showing the blue reaction product from the ammonium ferrous sulphate. The needle tract is clearly visible on the left (front), penetrating the zona tuberalis into the pars distalis. D) Sagittal section (20µm thick) through the pars distalis (stained with Cresyl violet) showing the diffusion area of the ammonium ferrous sulphate infusion (1µl/min) after 1h. OC optic chiasm, PD pars distalis, PN pars nervosa, PT pars tuberalis, ST sella turcica, ZT zona tuberalis;; Bar 15mm in A, 10mm in B–D

Figure 4.

Prolactin profiles before and after needle insertion and during saline infusion showing the percentage change in prolactin relative to baseline ($n=19$, $\text{mean} \pm \text{SEM}$). Individual ewes that responded to either the needle (dotted lines) or saline (dashed lines) are also shown.

Figure 5.

Prolactin profiles (percentage change in prolactin relative to baseline mean; mean±SEM) before, during and after TRH infusion. Representative prolactin profiles are shown on the left and mean profiles on the right. The washout period after cessation of TRH infusion was not monitored in all ewes as indicated by different n-values.

Figure 6. Prolactin profiles (percentage change in prolactin relative to baseline mean; mean $\pm$ SEM; n=3) comparing intra-pituitary with intravenous TRH infusions.

Figure 7.

Representative LH profiles (percentage change in LH relative to baseline mean) for 3 ewes before and during needle insertion and saline infusion (A) and during TRH infusion (B). Ewe 6308 showed a prolactin response to needle insertion, while ewes 6208 and 7329 were chosen because they showed a strong prolactin response to TRH infusion but not needle insertion or saline infusion.