PROLACTIN RELEASE IN THE SHEEP*

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The recent development of radioimmunoassay for prolactin has extended the depth and extent of studies of factors affecting prolactin levels in mammals and has permitted long-term studies to be made of changes in blood prolactin levels in individual animals. Although ovine prolactin has been widely studied in relation to its structure (Li, 1972) and biological activity (see Wolstenholme & Knight, 1972) and has been extensively used as a reference material, the serum prolactin levels of the sheep have not been widely investigated. Many of the early studies involved measurements of single samples obtained by venepuncture, a stressful procedure now known to affect prolactin release in the sheep (Saji, 1967; Arai & Lee, 1967; Bryant & Greenwood, 1968; McNeilly, 1971; Davis, Reichert & Niswender, 1971). Although there are several excellent reviews on the control of prolactin secretion in mammals (Meites, 1966, 1970; Meites & Nicoll, 1966; Pasteels, 1970; Nicoll, 1971; Meites, Lu, Wuttke, Welsch, Negasawa & Quadri, 1972; Wolstenholme & Knight, 1972), the majority of the citations in these reviews deal with work using laboratory species, with a few references to work on farm animals, primarily goats and cows. However, since the sheep, like the pig and nursing cow, exhibits a strong lactationally-induced anoestrus, a major factor limiting their productivity, knowledge of the mechanism involved in lactation anoestrus and the possible implications of prolactin in these mechanisms is critical in attempts to shorten the generation interval in these species. In sheep, goats and cows, prolactin levels have been studied in relation to stage and level of lactation, and in sheep new evidence is available implicating prolactin in the control of progesterone secretion (Denamur, Martinet & Short, 1973). This evidence, together with that indicating very high rates of release of prolactin at parturition, during early lactation, and following suckling in sheep, illustrates the need for more information concerning the mechanisms controlling prolactin release and the relationship between the synthesis and release of prolactin and those of FSH and LH.

In mammals, a reciprocal relationship has been suggested between the

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secretion of prolactin and the secretion of FSH and LH such that when the secretion of the former is high (e.g. in the lactating animal), that of the latter two hormones may be decreased; that it was the intensity of the sucking or milking stimulus rather than the secretion of milk which inhibited FSH and LH release was emphasized by Meites (1966). There is also evidence that when prolactin secretion is inhibited, e.g. by implanting a small quantity of prolactin in the median eminence (Vogt & Meites, 1971; Meites, 1972) or by the injection of L-dopamine into the third ventricle (Kamberi, Mical & Porter, 1970, 1971a, b), then the secretion of FSH and LH is increased and the release of LH- and FSH-releasing factors and prolactin-inhibiting factor (PIF) by the hypothalamus is increased (Kamberi *et al.*, 1971c).

These observations suggest that prolactin, in addition to exerting control of its own secretion by a 'short' feedback loop, may also play a rôle in regulating that of FSH and LH. Additional interest in prolactin release has centred around the concept that there may be a prolactin-releasing factor (PRF) in addition to the well-recognized PIF, and there is an increasing body of information which suggests that prolactin as well as ACTH may be released as a result of physical and psychological stress, since injury or disturbance is known to cause release of both hormones (Meites & Clemens, 1972).

Our experiments have been concerned with the long-term effects of season, pregnancy and lactation on prolactin levels and the influences of parturition and suckling on short-term changes in circulating prolactin. The studies were originally designed to investigate the possibility that the low fertility of some species which breed naturally during lactation (e.g. cattle), and of others which can be induced to breed during lactation (e.g. sheep), could be attributed to the adverse effects of prolactin secretion on gonadotrophin secretion and/or release. More recently, we have been concerned with the short-term influences of administering thyrotrophin-releasing factor (TRF) on plasma levels of prolactin. This work has been carried out in parallel with studies of the influence of LH-RF on the release of LH in the intact ewe (Crighton & Foster, 1972; Foster & Crighton, 1973) which has allowed comparisons to be made of the patterns of release of LH and prolactin following respectively the administration of LH-RF and TRF.

The radioimmunoassay procedure

A double antibody radioimmunoassay technique has been used for the majority of the prolactin measurements. Ovine prolactin (NIH-P-S6, 24.8 i.u./mg) has been used for antibody production, for radioiodination and as a standard (McNeilly, 1970). The prolactin antisera were raised in rabbits using Freund's complete adjuvant. Prolactin was labelled with sodium [125 I]iodide using the chloramine T method of Greenwood, Hunter & Glover (1963), and the specific activity usually ranged from 80 to 140 μ Ci/ μ g. In all assays, donkey anti-rabbit precipitating serum (Burroughs Wellcome MR66) was used as the second antibody and when using the assay routinely the working range extended from 30 pg/ml to 6 ng/ml. In experiments involving the addition of unlabelled prolactin to hypophysectomized sheep serum, recoveries ranged from 95.4% for 50 ng prolactin to 111.4% when 5 ng prolactin was added.

In specificity studies, there was no cross-reaction with ovine FSH (NIH-FSH-S9), ovine TSH (NIH-TSH-S6) or ovine LH (NIH-LH-S17). Cross-reaction was observed with ovine growth hormone (NIH-GH-S10), but the growth hormone preparation was shown to contain a substantial quantity of prolactin (A. S. McNeilly, personal communication) and there was no inhibition of the reaction between labelled ovine prolactin and its antiserum when concentrations as high as 100 $\mu\text{g}/\text{tube}$ of a purified preparation of growth hormone were used.

The effect of pregnancy and lactation

The first experiments involved comparisons of jugular plasma prolactin in sheep during pregnancy and lactation. For this experiment, a solid-phase system of radioimmunoassay was used (McNeilly, 1971). The blood samples were taken weekly by venepuncture from nine sheep; six pregnant and three non-pregnant. The animals were mated in the autumn and lambed in the spring; the measurements were made weekly from October to June. Plasma prolactin in the pregnant sheep remained low, between 10 and 50 ng/ml, until 6 weeks before parturition when higher levels were observed, averaging approximately 100 ng/ml but up to 500 ng/ml in some sheep. The levels remained high until parturition although there was a wide variation between animals particularly at 2 weeks before parturition. Following parturition, the plasma prolactin levels declined quite sharply, remaining high for only the 1st week of lactation until, at approximately 4 weeks *post partum*, baseline values were reached, although the animals continued to lactate for several weeks after this. The results indicating a sharp decline in prolactin during early lactation were similar to those obtained previously by Saji (1967) who used the haemagglutination-inhibition assay for measuring prolactin. These results differ substantially from those reported by Arai & Lee (1967) who used a double antibody radioimmunoassay for measuring serum prolactin in samples where the method of collection was not specified. In their work, higher serum values were obtained in early pregnancy and for an extended period of up to 3 months *post partum* during lactation. In the light of later information concerning the stress of the sampling procedure, it is possible that the results of Arai & Lee (1967) may have been influenced by stress of the animals during sampling. While samples obtained from trained sheep by venepuncture may give an indication of long-term trends in prolactin levels, little emphasis can be placed on the values obtained from single samples collected by venepuncture, especially if the animals are not handled frequently.

During late pregnancy in trained sheep sampled by way of a jugular catheter, a rise in prolactin is usually observed 1 to 2 days before parturition. As parturition commences, spurts of prolactin are released with high peaks as the lambs are born, levels reaching 700 ng/ml in some sheep. Undoubtedly, this pattern of release contributes to the high basal levels of prolactin during this phase. Immediately after parturition, the basal level of prolactin fluctuated between 100 and 200 ng/ml but, during the 1st week of lactation, a substantial spurt of prolactin is usually released at suckling.

By mid-lactation, the basal level was 20 to 100 ng/ml and suckling caused

a rise in prolactin from 20 to 400 ng. By late lactation, basal levels were 5 to 20 ng/ml (equivalent to the level in non-lactating anoestrous ewes), rising to 30 ng/ml with suckling. A comparison of samples collected early and later in lactation, therefore, show distinct differences in the basal levels and the extent of release due to suckling (McNeilly, Moseley & Lamming, 1972). These observations confirm that in the sheep there is a decline in prolactin levels as lactation advances, but the decline is more rapid than would be anticipated from the known pattern of lactation. This observation is similar to those made with machine milking in the cow (Johke, 1970) and hand milking in the goat (Hart, 1972) which show that artificial stimulation of the mammary gland produces similar patterns of release to those induced by the sucking stimulus.

TEXT-FIG. 1. Plasma prolactin levels measured daily throughout the oestrous cycle in three ewes. The arrows represent the occurrence of oestrus.

Prolactin levels during the oestrous cycle and oestrus

Although there are several reports concerning prolactin levels at oestrus (Bryant & Greenwood, 1968; Davis *et al.*, 1971; Cumming, Brown, Goding, Bryant & Greenwood, 1972), information is lacking about levels during the remainder of the cycle. Prolactin levels were determined in daily samples obtained by venepuncture from ewes during the breeding season. Text-figure 1 illustrates the results obtained in three sheep which were exhibiting cycles of normal length. The basal level of prolactin between oestrous periods was equivalent to levels in anoestrous ewes but each animal showed a characteristic rise in prolactin on the day of oestrus. Ten ewes were fitted with jugular vein catheters and blood samples were taken at 2-hr intervals from the start of oestrus. Considerable variation occurred between sheep in the levels of prolactin during oestrus and in the timing and pattern of the prolactin peaks which were observed. This variation is illustrated in Text-fig. 2; Sheep 2a and Sheep 2b had mid-oestrous peaks of 40 and 380 ng/ml, respectively, and Sheep 2c exhibited a peak 24 hr after the beginning of oestrus whereas Sheep 2d did not exhibit a measureable peak throughout the oestrous period. This type of variability is similar to that noted by Cumming *et al.* (1972) where

the timing of prolactin release was compared to the onset of the LH surge. Cumming *et al.* (1972) postulated that there may be a common mechanism stimulating the release of LH and prolactin at oestrus, drawing attention to the effect observed following oestradiol administration in anoestrous ewes.

TEXT-FIG. 2. Plasma prolactin levels in four ewes (Sheep 2a, 2b, 2c and 2d) during oestrus.

Table 1. Jugular vein prolactin levels in the sheep

<i>Reproductive status</i>	<i>Basal values</i>	<i>Peak response</i>
Seasonal anoestrous or cyclic ewes	5 to 20	Oestrous peak 40 to 400
Early pregnancy	10 to 20	
Late pregnancy	50 to 100	Parturition 800
Early lactation	100 to 150	Suckling 500
Mid-lactation	5 to 25	Suckling 400
Late lactation	5 to 20	Suckling 30

Prolactin levels are expressed in ng/ml plasma.

Table 1 summarizes the measurements of prolactin levels in the sheep and shows that there are major increases in the circulating levels of prolactin during late pregnancy and early lactation. In addition to the well documented effect of stress on prolactin release, peak responses have been obtained during oestrus,

as a result of mating, during parturition and first suckling with a characteristic decline in the response to sucking as lactation proceeds.

SHORT-TERM CHANGES IN CIRCULATING LEVELS OF PROLACTIN

In addition to the long-term changes in plasma prolactin levels, samples taken at frequent intervals have demonstrated that rapid fluctuations in the circulating levels of prolactin can occur over short periods. We have been interested in studying the pattern of these changes in response to non-specific stress, parturition, the suckling stimulus and the administration of a synthetic TRF.

Stress

Stress causes an elevation in the levels of prolactin in a number of species including the rat, sheep, goat, cow and human (Grosvenor, McCann & Naller,

TEXT-FIG. 3. Plasma prolactin levels in samples collected by syringe from a jugular venous cannula in a lactating ewe following intravenous infusion of 1 ml saline (S), 25 milliunits oxytocin (O) or 5 μ g adrenalin (A) in 1 ml saline.

1965; Bryant, Linzell & Greenwood, 1970; Johke, 1970; Neill, 1970; Butler, 1971; Raud, Kiddy & Odell, 1971; Cumming *et al.*, 1972; Frantz, Kleinberg & Noel, 1972; Fell, Findlay, Cumming & Goding, 1973). More specifically, the method of blood collection can affect the levels of prolactin. In the cow, Raud *et al.* (1971) found higher levels of prolactin when blood was collected by repeated jugular punctures than by withdrawal from an indwelling jugular catheter.

The first method of sampling used in this series of short-term experiments was to withdraw blood with a syringe from an indwelling jugular catheter. By this method, the frequency varied between one sample/min to one/5 min with 2 ml blood extracted by quickly attaching a fresh syringe to the catheter. From samples collected in this way, two observations were made. First, the levels of prolactin in the initial samples were usually elevated when

compared to later control values even though the animals had been accustomed to handling and to the sampling procedure for a period of weeks and seemed unafraid. Similar observations were made by Johke (1970) in the cow. The second observation was that in several trained sheep the injection of 1 ml physiological saline at 37°C into the jugular catheter also produced an apparent release of prolactin (Text-fig. 3) and it is suggested that the sheep was aware of the extra movements of the operator necessary for this procedure.

Because of these observations, we have concluded that results based on single samples, collected either by venepuncture or from a catheter, must be considered with caution and that studies on the effects of the injection of exogenous compounds on prolactin release must always be carefully controlled.

TEXT-FIG. 4. Diagram of the method of collection of blood samples from ewes using battery-operated roller pumps.

In order to minimize stress, a sampling system was developed so that blood could be removed without physical contact between the experimenter and the animal. An indwelling jugular catheter was connected to a battery-operated roller pump (Holter Precision Roller Pumps, Extracorporeal Medical Specialties, Inc., Pennsylvania 19406, U.S.A.) which was strapped to the animal's back. The blood passed down a length of polythene tubing into the sample tubes at a remote position outside the pen, and thus no restraint of the animal was necessary (Text-fig. 4). Initial investigations demonstrated that blood flow through the system was linear and that the rate of flow could be varied. A rate of 1 ml blood/30 sec was found convenient so that samples could be taken regularly and more frequently by this method than by a syringe. This system also enabled blood to be collected simultaneously from more than one site and substances could be infused simultaneously into the animal.

Text-figures 6(a) and 7(a) show that the rapid infusion of 1 ml saline over a 10-sec period did not produce any change in the basal level of prolactin. The normal basal level of prolactin measured from samples collected every 30 sec over a period of 30 to 50 min appears to show a pulsatile release with a variation in the level of prolactin between 2 and 25 ng/ml plasma. Recently, Mortimer, Besser, McNeilly & Goldie (1973) have noted pulsatile surges in the levels of plasma FSH and LH under basal conditions when samples were taken at 1-min intervals.

Spontaneous bursts of prolactin release have also been observed in the ewe by Fell, Beck, Brown, Catt, Cumming & Goding (1972). Their measurements, however, were on a different time scale since hourly samples were taken over a 40-hr period.

In reviewing the sampling techniques used in various experiments, it is important to have details of the placement of the jugular catheter because this can affect the absolute values of prolactin obtained. In the sheep, pituitary venous effluent can pass either through the external jugular veins or through vertebral vessels which join the caudal section of the superior vena cava. In our experiments, we have attempted to ensure that the tip of the catheter passes beyond the junction of the vertebral vessels; this was achieved by the use of catheters of a standard 30-cm length.

Parturition

An increase in the levels of prolactin at the time of parturition has been noted in several species including the rat, sheep, goat and cow (Arai & Lee, 1967; Bryant & Greenwood, 1968; Amenomori, Chen & Meites, 1970; Davis *et al.*, 1971; McNeilly, 1971; Buttle & Forsyth, 1971; Ingalls, Hafs & Oxender, 1971; Fell *et al.*, 1972). Because sampling has generally been infrequent in these experiments, however, the actual pattern of release during the period of delivery has not been recorded. An increase in the levels of prolactin about 2 days before parturition was noted by Ingalls *et al.* (1971) and Fell *et al.* (1972). It is tempting to associate this with the rapid rise in free circulating oestrogens and the decrease in progesterone that takes place at this time (Challis, 1971; Obst & Seamark, 1972; Chamley & co-authors, 1973; Robertson & Smeaton, 1973), but a careful study of the time sequence of these events is necessary before any more definite conclusions can be drawn.

In our studies, samples were taken at daily intervals before parturition by withdrawing blood with a syringe from an indwelling jugular catheter. Once labour started and uterine contractions were frequent, sequential samples were taken at intervals between 1 min and 5 min and continued until after the first suckling period (Lamming, Moseley & McNeilly, 1972).

In three of the five ewes studied, there was a small rise in the level of prolactin during the 48 hr preceding parturition. During the final stages of labour and at parturition, rapid spurt releases of prolactin took place and jugular levels were over 600 ng/ml plasma in some animals. Following parturition, the level of prolactin fell rapidly and remained low during the period that the lamb was attempting to reach the udder until at first suckling there was again a dramatic increase, and values of over 800 ng/ml plasma were recorded

in some animals. After suckling, the levels of prolactin rapidly declined to a baseline (see Text-figs 5a and b).

Suckling

It is well established that suckling results in a release of prolactin, as recently demonstrated in the rat, sheep, goat, cow and human (Bryant & Greenwood, 1968; Amenomori *et al.*, 1970; Karg & Schams, 1970; Buttle & Forsyth, 1971; Fell & co-authors, 1971; Koprowski, Convey & Tucker, 1971; Frantz *et al.*, 1972; Tyson, Friesen & Anderson, 1972; Fell *et al.*, 1972; Buttle, Forsyth &

TEXT-FIG. 5. Plasma prolactin levels of (a) Ewe 487, and (b) Ewe 690 during parturition and first suckling. L, lamb delivered; n, nuzzling by lamb; s, sucking by lamb.

Knaggs, 1972; Gautvik, Weintraub, Graeber, Maloof, Zuckerman & Tashjian, 1973).

Sampling has generally been infrequent, however, and it has not been possible to follow the pattern of release during suckling. By collecting samples using a remote-control pump, it was possible to measure the prolactin level at 30-sec intervals throughout the suckling period. Text-figure 8 shows the release of prolactin in a ewe 1 week *post partum* suckling one lamb. Blood was also collected simultaneously from the venous ring around the base of the udder, by an indwelling catheter inserted caudally in the subcutaneous abdominal vein which was occluded at the point of entry. The prolactin levels in the venous effluent from the mammary gland did not show a comparable rise found in jugular venous blood during the suckling period. It is interesting that prolactin levels in mammary venous blood rose and followed a similar pattern to that in jugular blood when prolactin release was stimulated by the exogenous administration of TRF. (Text-figs 7b, c and d).

There was considerable variation in the release of prolactin during suckling between sampling periods for the same ewe. This was probably because the unrestrained presence of the lamb is essential with lactating animals in order to avoid stress so that the suckling interval is variable. Grosvenor & Mena (1971) have reported the existence of a refractory period in the release of prolactin after suckling in the rat.

Administration of thyrotrophin-releasing factor

There is wide acceptance that TRF (pyro-glu-his-pro-NH₂) is the physiological releasing factor for thyroid-stimulating hormone (TSH). The concept of the specificity of the interaction between TRF and its target organ was questioned when Tashjian, Barowsky & Jensen (1971) reported that TRF stimulated production and release of prolactin in clonal strains of rat pituitary cells in culture. This was not confirmed by Lu, Shaar, Kortright & Meites (1972) using both normal male pituitary halves and rat pituitary tumour cells. More recently, Vale, Blackwell, Grant & Guillemin (1973) could only demonstrate a release of prolactin in response to TRF from cell cultures and half pituitaries from rats fed propylthiouracil.

A single injection of 5.0 or 7.5 µg TRF produced no effect on serum prolactin in the rat *in vivo*, but six daily injections of 50 µg TRF resulted in a small increase (Lu *et al.*, 1972). Deis & Alonson (1973), however, found a significant increase in plasma prolactin levels in female rats in response to 0.5 and 1.0 µg TRF.

The ability of TRF to release prolactin *in vivo* in other species does not appear to be so equivocal and has been demonstrated in the sheep, cow and human (Bowers, Friesen, Hwang, Guyda & Folkers, 1971; Jacobs, Snyder, Wilbur, Utiger & Daughaday, 1971; L'Hermite & co-authors, 1972; Convey, Tucker, Smith & Zolman, 1972; Fell *et al.*, 1973; Gautvik *et al.*, 1973).

We have examined the pattern of prolactin release in response to single intravenous injections of synthetic TRF (Hoechst Pharmaceuticals) in lactating and anoestrous ewes. Samples were collected at the rate of 1 ml/30 sec over 30 to 50 min. Text-figures 6(a), 6(b) and 6(c) show a typical series of samples

collected from a lactating ewe 4 weeks *post partum*. The intravenous injection of 1 ml saline over a 10-sec period as a control test did not cause a release of prolactin and the basal levels varied between 2 and 25 ng/ml (Text-fig. 6a). The administration of 250 μ g TRF in 1 ml saline, however, resulted in a rapid rise in prolactin levels, with a further peak of prolactin reaching 84 ng/ml between 18 and 25 min after the administration of TRF (Text-fig. 6b). During the last 12 min of sampling, the level of prolactin returned to the baseline. The administration of 250 μ g of another tripeptide, pyro-glutamyl-valyl-seryl-amide did not cause a release of prolactin (Text-fig. 6c). A similar pattern of release was obtained in anoestrous ewes (Text-fig. 6d).

TEXT-FIG. 6. Plasma prolactin levels in (a) a non-suckling lactating ewe, 4 weeks after lambing; (b) a lactating ewe after an intravenous infusion of 250 μ g thyrotrophin-releasing factor (TRF); (c) a lactating ewe after an intravenous infusion of 250 μ g of a tripeptide (pyro-glu-val-ser-NH₂); (d) a non-lactating ewe after an intravenous infusion of 250 μ g TRF. The results shown in a, b and c are from the same ewe.

A rapid and prolonged response to TRF was obtained in one lactating ewe during the first 2 weeks *post partum* (Text-figs 7a, 7b, 7c and 7d). Following the administration of 120 μ g TRF, the level of prolactin began to rise within 2 min and the response curve was characterized by successive bursts of prolactin release reaching a peak of 150 ng/ml by 8 min. Prolactin remained elevated up to the end of the sampling period 35 min after the administration of TRF. The peak response was not related directly to dose level between 120 μ g and 480 μ g (Text-figs 7b, 7c and 7d). Mammary venous levels showed a similar rise unlike the response observed due to the suckling stimulus (Text-fig. 8).

The administration of 150 μ g synthetic decapeptide assumed to be LH-RF (Hoechst Pharmaceuticals) had no effect on circulating prolactin levels in the anoestrous ewe and, conversely, the administration of 300 μ g TRF did not produce a rise in LH levels. It is interesting however, to compare the release

TEXT-FIG. 7. Plasma prolactin levels in a lactating ewe after intravenous infusion of (a) 1 ml saline; or 1 ml saline containing (b) 120 μ g TRF; (c) 240 μ g TRF; (d) 480 μ g TRF. ●, Jugular vein; ○, mammary vein.

patterns of prolactin and LH in the same anoestrous ewe in response to TRF and LH-RF respectively (Text-fig. 9). The level of prolactin began to rise within 20 min of administration of TRF, with a peak at 25 min, and had returned to the baseline by 45 min. The level of LH in response to LH-RF rose within

20 min but peak values were not obtained until 90 to 110 min, and levels had not reached a baseline by 180 min. Table 2 summarizes our results on the effect of TRF on prolactin and those of colleagues (Crichton & Foster, 1972; Foster & Crichton, 1973) on the effect of LH-RF on LH. Our results have shown that in the lactating ewe shortly after parturition the response to TRF may be even more rapid, levels of prolactin rising 2 min after administration. It is the rapidity with which prolactin is released that has led to the general conclusion that TRF acts directly as a releasing factor on the pituitary, although it may not be the physiological releasing factor. Although it has been shown both *in vitro* and *in vivo* in the rat that thyroid hormones can influence pituitary concentration

TEXT-FIG. 8. Plasma prolactin levels in a lactating ewe allowed to suckle (S). ●, Jugular vein; ○, mammary vein.

and secretion of prolactin (Nicoll & Meites, 1963; Chen & Meites, 1969), the latter would not appear to be an intermediary in the response to TRF. L'Hermite *et al.* (1972) have demonstrated in the human that while peak values of prolactin occurred as early as 5 to 10 min after TRF administration, the peaks of TSH occurred at 20 to 40 min. A similar differential response has been reported in the cow (Convey *et al.*, 1972). Recently, it has been reported that there is no elevation of TSH levels during suckling although prolactin levels reach a peak (Gautvik *et al.*, 1973; Kann *et al.*, 1973). In the human, no prolactin response to suckling was found during the later stages of lactation, but prolactin was released in response to TRF administered 5 to 15 min after a 30-min suckling period (Tyson *et al.*, 1972). Thus, although it

may be concluded that TRF acts to produce a rapid release of prolactin, there is, as yet, little evidence to suggest that it is the physiological releasing factor.

The rapidity with which the levels of circulating prolactin fluctuate has led to the proposition that a releasing-factor system does exist as well as the prolactin-inhibiting system. Benson & Folley (1956) suggested that oxytocin may be the hypothalamic factor responsible for prolactin release. Our failure in the

TEXT-FIG. 9. (a) Plasma prolactin levels in a ewe after intravenous infusion of 300 μ g TRF, and (b) plasma prolactin and LH values after intravenous infusion of 150 μ g LH-releasing factor (LHRF). ●, Prolactin; △, LH.

Table 2. Timing of pituitary response to releasing factors in the intact ewe

	<i>LH-RF</i> → <i>LH</i>	<i>TRF</i> → <i>Prolactin</i>
Injection to response (min)	20 to 40	2 to 18
Injection to peak (min)	90 to 120	5 to 25
Duration of response (min)	>210	7 to 45

LH-RF, LH-releasing factor; *TRF*, thyrotrophin-releasing factor.

course of many experiments to find any evidence that exogenous oxytocin is capable of releasing prolactin is in agreement with the findings of Bryant *et al.* (1970).

It is interesting to consider the physiological situations that occur where both oxytocin and prolactin are released, namely at parturition (see McNeilly & Hart, 1973), suckling and during mating, and a more detailed study of the

interrelationship between these two hormones would appear to be worthy of investigation. Although exogenous TRF is capable of releasing prolactin, we have already noted that TSH is not released during suckling and, furthermore, stress produces a rapid decline in the circulating levels of TSH (Ducommun, Sakiz & Guillemin, 1966). This suggests that TRF is not always released in physiological situations that cause the release of prolactin although the possibility exists that the endogenous levels of TRF required to release prolactin and TSH differ.

There is evidence from experiments *in vitro* for the existence of a prolactin-releasing factor (Nicoll, Fiorindo, McKenney & Parsons, 1970). Valverde, Chieffo & Reichlin (1972) claim that the intravenous injection of crude methanol or acid extracts of porcine hypothalamic fragments induce a significant rise in assayable prolactin within 10 min in male rats sensitized with oestrogen and progesterone. Amounts of TRF far in excess of those in the crude extract had no effect.

TEXT-FIG. 10. Distribution of intervals between peaks of prolactin release in ewes.

CONCLUSIONS

The method of blood collection by the remote-control sampling device has allowed the pattern of prolactin release in the sheep to be studied in some detail. All the records of samples taken at 30-sec intervals were examined, and all peaks of 5 ng or more were noted, and the interval in minutes between peaks was calculated. These values were then analysed statistically by an analysis of variance to determine whether there was a difference in the pulsatile pattern

of release between individual animals and also whether treatment (suckling or the administration of TRF) affected this pattern. This analysis revealed no significant differences between individual sheep, between control and treatment sequences or between basal levels and response periods (taken from suckling or injection of TRF until prolactin returned to basal values) within the same sequence. Analysis of 112 peaks from nine sequences revealed a mean interval between peaks of 2.93 min with a standard deviation of individual peaks of 1.20 min and a distribution of peaks as shown in Text-fig. 10. We conclude, therefore, that prolactin is normally released in a pulsatile form in the sheep and that the responses to the suckling stimulus or the injection of TRF lead to higher peaks of release at a similar frequency.

From the foregoing observations, we feel that because stress precipitates the release of prolactin, the use of a remote-control sampling system is essential for the study of short-term changes in levels of this hormone. By this method, repeated samples can be taken at brief intervals.

A highly significant release of prolactin was noted at parturition, especially at delivery and at first suckling; thereafter, the response to the suckling stimulus declined. In lactating and anoestrous sheep, the intravenous administration of TRF resulted in a rapid release of prolactin, but there is still no definite evidence that TRF is the physiological releasing factor for prolactin.

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REFERENCES

- AMENOMORI, Y., CHEN, C. L. & MEITES, J. (1970) Serum prolactin levels in rats during different reproductive states. *Endocrinology*, **86**, 506.
- ARAI, Y. & LEE, T. H. (1967) Double antibody radioimmunoassay procedure for ovine pituitary prolactin. *Endocrinology*, **81**, 1041.
- BENSON, G. K. & FOLLEY, S. J. (1956) Oxytocin as stimulator for the release of prolactin from the anterior pituitary. *Nature, Lond.* **177**, 700.
- BOWERS, C. Y., FRIESEN, H. G., HWANG, P., GUYDA, H. J. & FOLKERS, K. (1971) Prolactin and thyrotrophin release in man by synthetic pyro-glutamyl-histidyl-prolinamide. *Biochem. biophys. Res. Commun.* **45**, 1033.
- BRYANT, G. D. & GREENWOOD, F. C. (1968) Radioimmunoassay for ovine, caprine and bovine prolactin in plasma and tissue extracts. *Biochem. J.* **109**, 831.
- BRYANT, G. D., LINZELL, J. F. & GREENWOOD, F. C. (1970) Plasma prolactin in goats measured by radioimmunoassay. The effects of teat stimulation, mating behaviour, stress, fasting and oxytocin, insulin and glucose injections. *Hormones*, **1**, 26.
- BUTLER, W. R. (1971) Patterns of release of ovine prolactin. *J. Anim. Sci.* **33**, 250, Abstr.
- BUTTLE, H. L. & FORSYTH, I. A. (1971) Prolactin concentration and lactogenic activity in the plasma of lactating and pregnant goats. *J. Endocr.* **51**, xxxiii.
- BUTTLE, H. L., FORSYTH, I. A. & KNAGGS, G. S. (1972) Plasma prolactin measured by radioimmunoassay and bioassay in pregnant and lactating goats and the occurrence of a placental lactogen. *J. Endocr.* **53**, 483.
- CHALLIS, J. R. G. (1971) Sharp increase in free circulating oestrogens immediately before parturition in the sheep. *Nature, Lond.* **229**, 208.
- CHAMLEY, W. A., BUCKMASTER, J. M., CERINI, M. E., CUMMING, I. A., GODING, J. R., OBST, J. M., WILLIAMS, A. & WINFIELD, C. (1973) Changes in the levels of progesterone, corticosteroids, estrone, estradiol-17 β , luteinizing hormone and prolactin in the peripheral plasma of the ewe during late pregnancy and parturition. *Biol. Reprod.* **9**, 30.
- CHEN, C. L. & MEITES, J. (1969) Effects of thyroxine and thiouracil on hypothalamic PIF and pituitary prolactin levels. *Proc. Soc. exp. Biol. Med.* **131**, 576.

- CONVEY, E. M., TUCKER, H. A., SMITH, U. G. & ZOLMAN, J. (1972) Prolactin, thyroxine and corticoid after TRH. *J. Anim. Sci.* **35**, 258, Abstr.
- CRIGHTON, D. B. & FOSTER, J. P. (1972) The effect of a synthetic preparation of gonadotrophin-releasing factor on gonadotrophin release from the ovine pituitary *in vitro* and *in vivo*. *J. Endocr.* **55**, xiii.
- CUMMING, I. A., BROWN, J. M., GODING, J. R., BRYANT, G. D. & GREENWOOD, F. C. (1972) Secretion of prolactin and luteinising hormone at oestrus in the ewe. *J. Endocr.* **54**, 207.
- DAVIS, S. L., REICHERT, L. E. & NISWENDER, G. D. (1971) Serum levels of prolactin in sheep as measured by radioimmunoassay. *Biol. Reprod.* **4**, 145.
- DEIS, R. P. & ALONSON, N. (1973) Prolactin release induced by synthetic thyrotrophin releasing factor in female rats. *J. Endocr.* **58**, 673.
- DENAMUR, R., MARTINET, J. & SHORT, R. V. (1973) Pituitary control of the ovine corpus luteum. *J. Reprod. Fert.* **32**, 207.
- DUCOMMUN, P., SAKIZ, E. & GUILLEMIN, R. (1966) Lability of plasma TSH levels in the rat in response to non-specific exteroceptive stimuli. *Proc. Soc. exp. Biol. Med.* **121**, 921.
- FELL, L. R., BECK, C., BLOCKLEY, M. A. DE B., BROWN, J. M., CATT, K. J., CUMMING, I. A. & GODING, J. R. (1971) Prolactin in the dairy cow during suckling and machine milking. *J. Reprod. Fert.* **24**, 144.
- FELL, L. R., BECK, C., BROWN, J. M., CATT, K. J., CUMMING, I. A. & GODING, J. R. (1972) Solid-phase radioimmunoassay of ovine prolactin in antibody-coated tubes. Prolactin secretion during estradiol treatment, at parturition, and during milking. *Endocrinology*, **91**, 1329.
- FELL, L. R., FINDLAY, J. K., CUMMING, I. A. & GODING, J. R. (1973) Effect of synthetic TRF on prolactin release in the sheep. *Endocrinology*, **93**, 487.
- FOSTER, J. P. & CRIGHTON, D. B. (1973) Preliminary observations on the administration of a synthetic preparation of gonadotrophin releasing factor to cyclic and anoestrous ewes. *J. Endocr.* **57**, xxv.
- FRANTZ, A. G., KLEINBERG, D. L. & NOEL, G. L. (1972) Studies on prolactin in man. *Recent Prog. Horm. Res.* **28**, 527.
- GAUTVIK, K. M., WEINTRAUB, B. D., GRAEBER, C. T., MALOOF, F., ZUCKERMAN, J. E. & TASHJIAN, A. H. (1973) Serum prolactin and TSH: effects of nursing and pyro-glu-His-Pro-NH₂ administration in *post-partum* women. *J. clin. Endocr. Metab.* **37**, 135.
- GREENWOOD, F. C., HUNTER, W. M. & GLOVER, J. S. (1963) The preparation of ¹³¹I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89**, 114.
- GROSVENOR, C. E., MCCANN, S. M. & NALLER, R. (1965) Inhibition of nursing-induced and stress-induced fall in pituitary prolactin concentration in lactating rats by injection of acid extracts of bovine hypothalamus. *Endocrinology*, **76**, 883.
- GROSVENOR, C. E. & MENA, F. (1971) Evidence for a refractory period in the neuroendocrine mechanism for the release of prolactin. *Endocrinology*, **88**, 355.
- HART, I. C. (1972) Level of prolactin in the blood of the goat during milking, throughout lactation and over a 24-hr period. *J. Endocr.* **55**, xxviii.
- INGALLS, W., HAFS, H. D. & OXENDER, W. D. (1971) Growth hormone, prolactin and luteinising hormone in heifers before and after parturition. *J. Dairy Sci.* **54**, 786, Abstr.
- JACOBS, L. S., SNYDER, P. J., WILBER, J. F., UTIGER, R. D. & DAUGHADAY, W. H. (1971) Increased serum prolactin after administration of synthetic thyrotropin releasing hormone (TRH) in man. *J. clin. Endocr. Metab.* **33**, 996.
- JOHKE, T. (1970) Factors affecting plasma prolactin level in the cow and the goat as determined by radioimmunoassay. *Endocr. jap.* **17**, 393.
- KAMBERI, I. A., MICAL, R. S. & PORTER, J. C. (1970) Effect of anterior pituitary perfusion and intraventricular injection of catecholamines and indoleamines on LH release. *Endocrinology*, **87**, 1.
- KAMBERI, I. A., MICAL, R. S. & PORTER, J. C. (1971a) Effects of anterior pituitary perfusion and intraventricular injection of catecholamines on FSH release. *Endocrinology*, **88**, 1003.
- KAMBERI, I. A., MICAL, R. S. & PORTER, J. C. (1971b) Effect of anterior pituitary perfusion and intraventricular injection of catecholamines on prolactin release. *Endocrinology*, **88**, 1012.
- KAMBERI, I. A., MICAL, R. S. & PORTER, J. C. (1971c) Hypophyseal portal vessel infusion: *in vivo* demonstration of LRF, FRF and PIF in pituitary stalk plasma. *Endocrinology*, **89**, 1042.
- KANN, G., HABERT, R. & DENAMUR, R. (1973) Concentrations plasmatiques de la prolactine et de l'hormone thyroïdostimulante au cours de la traite des brebis. Comparaisons les effets du TRF. *C. r. hebdom. Séanc. Acad. Sci., Paris*, **276**, 1321.
- KARG, H. & SCHAMS, D. (1970) Discussion on prolactin levels in bovine blood under different physiological conditions. In *Lactation*, p. 141. Ed. I. R. Falconer. Butterworths, London.
- KOPROWSKI, J. A., CONVEY, E. M. & TUCKER, H. A. (1971) Response of serum prolactin to stimulation of the udder or brisket. *J. Dairy Sci.* **54**, 769, Abstr.
- LAMMING, G. E., MOSELEY, S. R. & MCNEILLY, J. R. (1972) Prolactin release in the ewe at parturition and first suckling. *J. Endocr.* **55**, xxvii.

- L'HERMITE, M., VANHAELST, L., COPINSCH, I. G., LECLERQ, Q. R., GOLDSTEIN, J., BRUNO, O. D. & ROBYN, C. (1972) Prolactin release after injection of thyrotrophin releasing hormone in man. *Lancet*, **i**, 763.
- LI, C. H. (1972) Recent knowledge of the chemistry of lactogenic hormones. In *Lactogenic Hormones*, pp. 7-26. Eds. G. E. W. Wolstenholme and J. Knight. Churchill Livingstone, Edinburgh.
- LU, K. H., SHAAH, C. J., KORTRIGHT, & MEITES, J. (1972) Effects of synthetic TRH on *in vitro* and *in vivo* prolactin release in the rat. *Endocrinology*, **91**, 1540.
- MCNEILLY, A. S. & HART, I. C. (1973) The release of oxytocin and prolactin during parturition in the goat. *J. Endocr.* **56**, 159.
- MCNEILLY, J. R. (1970) *Studies on the development of a radioimmunoassay for ovine prolactin*. Ph.D. thesis, University of Nottingham.
- MCNEILLY, J. R. (1971) A solid-phase radioimmunoassay for ovine prolactin. *J. Endocr.* **49**, 141.
- MCNEILLY, J. R., MOSELEY, S. R. & LAMMING, G. E. (1972) Observations on the pattern of prolactin release during suckling in the ewe. *J. Reprod. Fert.* **31**, 487.
- MEITES, J. (1966) Control of mammary growth and lactation. In *Neuroendocrinology*, Vol. 1, p. 669. Eds. L. Martini and W. F. Ganong. Academic Press, New York.
- MEITES, J. (1970) Direct studies of the secretion of the hypothalamic hypophysiotropic hormones (HHH). In *Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry*, p. 261. Ed. J. Meites. Williams & Wilkins, Baltimore.
- MEITES, J. (1972) Hypothalamic control of prolactin secretion. In *Lactogenic Hormones*, pp. 325-338. Eds. G. E. W. Wolstenholme and J. Knight. Churchill Livingstone, Edinburgh.
- MEITES, J. & CLEMENS, J. A. (1972) Hypothalamic control of prolactin secretion. *Vitam. Horm.* **30**, 165.
- MEITES, J., LU, K. H., WUTTKE, W., WELSCH, C. W., NEGASAWA, H. & QUADRI, S. K. (1972) Recent studies on functions and control of prolactin secretion in rats. *Recent Prog. Horm. Res.* **28**, 471.
- MEITES, J. & NICOLL, C. S. (1966) Adenohypophysis: prolactin. *A. Rev. Physiol.* **28**, 57.
- MORTIMER, G. H., BESSER, G. M., MCNEILLY, A. S. & GOLDIE, D. J. (1973) Asynchronous pulsatile luteinizing hormone and follicle-stimulating hormone responses during luteinizing hormone-follicle-stimulating hormone releasing hormone and thyroid-stimulating hormone releasing hormone infusions. *J. Endocr.* **59**, xii.
- NEILL, J. D. (1970) Effect of stress on serum prolactin and luteinizing hormone levels during the oestrous cycle in the rat. *Endocrinology*, **87**, 1192.
- NICOLL, C. S. (1971) Aspects of the neural control of prolactin secretion. In *Frontiers in Neuroendocrinology* pp. 291-330. Eds. L. Martini and W. F. Ganong. Oxford University Press, London and New York.
- NICOLL, C. S., FIORINDO, R. P., MCKENNEE, C. T. & PARSONS, J. A. (1970) Assay of hypothalamic factors which regulate prolactin secretion. In *Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry*, p. 115. Ed. J. Meites. Williams & Wilkins, Baltimore.
- NICOLL, C. S. & MEITES, J. (1963) Prolactin secretion *in vitro*: effects of thyroid hormones and insulin. *Endocrinology*, **72**, 544.
- OBST, J. M. & SEAMARK, R. F. (1972) Plasma oestrogen concentrations in ewes during parturition. *J. Reprod. Fert.* **28**, 161.
- PASTEELS, J. L. (1970) Control of prolactin secretion. In *The Hypothalamus*, p. 385. Eds. L. Martini, M. Motta and F. Fraschini. Academic Press, New York and London.
- RAUD, H. R., KIDDY, C. A. & ODELL, W. D. (1971) The effect of stress upon the determination of serum prolactin by radioimmunoassay. *Proc. Soc. exp. Biol. Med.* **136**, 689.
- ROBERTSON, H. A. & SMEATON, T. C. (1973) The concentration of unconjugated oestrone, oestradiol-17 α and oestradiol-17 β in the maternal plasma of the pregnant ewe in relation to the initiation of parturition and lactation. *J. Reprod. Fert.* **35**, 461.
- SAJI, M. (1967) An immunological estimation of prolactin in sheep blood. In *Reproduction in the Female Mammal*, pp. 157-176. Eds. G. E. Lamming and E. C. Amoroso. Butterworths, London.
- TASHJIAN, A. H., BAROWSKY, N. J. & JENSEN, D. K. (1971) Thyrotrophin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. *Biochem. biophys. Res. Commun.* **43**, 517.
- TYSON, J. E., FRIESEN, H. G. & ANDERSON, M. S. (1972) Human lactational and ovarian response to endogenous prolactin release. *Science, N.Y.* **177**, 897.
- VALE, W., BLACKWELL, R., GRANT, G. & GUILLEMIN, R. (1973) TRF and thyroid hormones on prolactin secretion by rat anterior pituitary cells *in vitro*. *Endocrinology*, **93**, 26.
- VALVERDE, R. C., CHIEFFO, V. & REICHLIN, S. (1972) Prolactin-releasing factor in porcine and rat hypothalamic tissue. *Endocrinology*, **91**, 982.
- VOGT, J. & MEITES, J. (1971) Effects of an implant of prolactin in median eminence of pseudopregnant rats on serum and pituitary LH, FSH and prolactin. *Endocrinology*, **88**, 286.
- WOLSTENHOLME, G. E. W. & KNIGHT, J. (Eds.) (1972) *Lactogenic Hormones*. Churchill Livingstone, Edinburgh.