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Regulation of the secretion of FSH in domestic ruminants

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Introduction

In the past 2–3 years there have been considerable advances in our understanding of the neuroendocrine processes controlling pituitary function (Clarke, 1987) and the nature of steroidal and non-steroidal feedback regulation, particularly by the gonadal peptide, inhibin (Findlay, 1986). These advances offer new insights into the regulation of gonadotrophin secretion and gonadal function, and could have a considerable commercial impact in animal industry. In this review, we summarize these advances with respect to the control of FSH secretion in sheep and cattle.

Patterns of FSH secretion

The main characteristics of the patterns of FSH concentration in peripheral plasma of sheep (L'Hermite *et al.*, 1972; Salamonsen *et al.*, 1973) and cows (Walters & Schallenberger, 1984; Quirk & Fortune, 1986) are (a) relatively low levels, compared to the luteal phase, of FSH during the 1–2 days before the preovulatory surge of gonadotrophins, i.e. when LH and oestradiol secretion are increasing (Baird & McNeilly, 1981), (b) a peak of FSH secretion coincident with the preovulatory surge of LH, both of which are induced by oestradiol (Jonas *et al.*, 1973; Kesner & Convey, 1982), (c) a second surge of FSH commencing 18–24 h after onset of the preovulatory release, reaching peak concentrations just after ovulation when LH and oestradiol concentrations are both low (Baird & McNeilly, 1981) and (d) fluctuating concentrations of FSH during the remainder of the cycle. It has been estimated that the major sources of variation in peripheral concentrations of FSH during the oestrous cycle of the ewe are attributable to animals (75%) and time of sampling (16% between days and 9% between hours) (Findlay & Cumming, 1976). Unlike LH, FSH secretion in sheep is not pulsatile (see Clarke *et al.*, 1986), whereas there is evidence of pulsatility in cattle (Walters & Schallenberger, 1984).

Measurements of metabolic clearance (MCR) of FSH (Akbar *et al.*, 1974; Findlay & Cumming, 1976) support the notion that the changes in peripheral concentrations of FSH are due to changes in secretion by the pituitary gland and not removal of hormone from blood.

In addition to the cyclic changes there are other factors known to influence FSH secretion. For example, in sheep the concentrations of FSH have been related to breed (Lahlou-Kassi *et al.*, 1984; Bindon *et al.*, 1985a, b), sex (Findlay *et al.*, 1985), nutrition (Scaramuzzi & Radford, 1983), season (Findlay & Cumming, 1976; Findlay *et al.*, 1985) and age (Lee *et al.*, 1976; Bindon *et al.*, 1985b). In all of these instances, and especially during the ovarian cycle, it is apparent that the secretion of FSH can change independently of LH secretion, indicating that the control systems regulating the two pituitary gonadotrophins are not necessarily the same. There is evidence that the control processes regulating FSH, which are independent of those controlling LH, reside at the level of the hypothalamus and the pituitary gland and in the nature of the feedback influences of the gonads.

Hypothalamic control of FSH secretion

Disconnection of the direct hypothalamic influence on the pituitary gland of sheep (Clarke *et al.*, 1983) and active immunization against gonadotrophin-releasing hormone (GnRH) (McNeilly *et al.*, 1986) leads to a cessation of the secretion of FSH and LH. In these models, FSH secretion continued but at a decreased rate for days whereas LH secretion had stopped within 48–72 h. Anaesthesia of ewes at the time of luteolysis delayed the fall in FSH and the rise in LH in plasma and delayed the onset of the preovulatory surges of both gonadotrophins (Radford *et al.*, 1978). The hypothalamic influence on pituitary FSH secretion involves GnRH, which will stimulate secretion of both LH and FSH *in vivo* in sheep (Jonas *et al.*, 1973) and cattle (Kesner & Convey, 1982). The existence of a specific FSH-releasing hormone has been postulated (Bowers *et al.*, 1973) to account for the differential secretion rates and patterns of FSH and LH, but there has been no firm evidence to support the hypothesis. Recently, however, Ying *et al.* (1986a) reported that one of the cell growth factors, transforming growth factor-β (TGF-β), caused a dose-dependent stimulation of FSH secretion by rat pituitary cells *in vitro* when added at only attomolar concentrations. Subsequently, 2 polypeptides related to inhibin have been isolated from pig follicular fluid which are potent and selective stimulators of FSH release *in vitro*. One has been called follicle-stimulating hormone regulatory protein and is a homodimer of M_r 28 000 composed of two β_A subunits of inhibin (Vale *et al.*, 1986). The other heterodimer is called activin and consists of a β_A and a β_B subunit of inhibin (Ling *et al.*, 1986). The inhibin subunits share considerable sequence homology with TGF-β, itself a homodimer (Derynck *et al.*, 1985). It will be necessary to establish whether or not the β-dimers and/or TGF-β are secreted by the gonads or are produced locally in the hypothalamic–pituitary unit. A lack of glycosylation sites on the β-subunits of inhibin (see below) which would render the β-dimers very susceptible to peripheral metabolism, and the potential activation of TGF-β sites in many different tissues by circulating β-dimers argues in favour of local production of β-dimers by the hypothalamus and/or pituitary rather than secretion by the gonads.

Control of FSH secretion by higher brain centres can be shown in gonadectomized animals. Peripheral FSH concentrations are higher in sheep in the breeding season than in seasonal anoestrus and both FSH and LH are higher in males than in females (Findlay *et al.*, 1985). While these influences are likely to be mediated by the hypothalamus, the neuroendocrine processes involved are not understood. They are likely to be functional in intact animals as well because circulating FSH concentrations are reported to be higher in breeding than in anoestrous sheep (Findlay & Cumming, 1976; Lincoln & Short, 1980), an observation not shared by all investigators, however (Walton *et al.*, 1977). The sex difference in peripheral gonadotrophin concentrations could reflect greater GnRH pulse frequency and amplitude, greater sensitivity of the pituitary to releasing hormone stimulation or lower clearance of FSH in the males. The pituitary content of FSH and LH in intact rams is only 10–20% of that in cyclic ewes (Robertson *et al.*, 1984), supporting the idea of higher release rates of FSH in males.

There is evidence for a difference between the secretion of LH and FSH due to an inherent property of pituitary cells. Removal of the pituitary cells from the influence of the hypothalamus (Clarke *et al.*, 1983) or GnRH *in vivo* (McNeilly *et al.*, 1986) or *in vitro* (Miller *et al.*, 1977) results in a slower decrease in the secretion rate of FSH compared to LH that cannot be accounted for by a difference in the MCR of the two glycoproteins. It appears that FSH, unlike LH, does not require the presence of GnRH, either intermittently or continuously, to be released by the gonadotrophs. It is not known whether this is a property of FSH or the type of gonadotroph, since there are cells in sheep and cattle pituitary glands which contain only FSH immunoreactivity as well as those with immunoreactivity for LH and FSH (P. Somogyi, I. W. Chubb, I. J. Clarke, J. K. Findlay & R. Fischer-Colbie, unpublished observations). The availability of complementary DNA (cDNA) probes coding for messenger RNA for the gonadotrophin subunits and methods to study the synthesis and secretions of hormones by individual pituitary cells should contribute to answering those questions.

Gonadal control of FSH secretion

It is well established that the gonads can influence the secretion of both FSH and LH due to the inhibitory effects of gonadal steroids (Goodman & Karsch, 1981). In the female, there is also a positive feedback effect of oestradiol on FSH and LH secretion (Jonas *et al.*, 1973; Kesner & Convey, 1982) which is responsible for the preovulatory surge of gonadotrophins during the ovarian cycle.

The feedback control of gonadotrophin secretion by steroids can be manifested at both the hypothalamic and pituitary level. Clarke (1987) has defined two types of negative feedback effect of oestrogen in sheep, in addition to the positive effect mentioned above. The short-term negative feedback effect is seen when ovariectomized ewes are treated with oestradiol (Clarke *et al.*, 1982). Both LH and FSH concentrations fall in peripheral plasma. The effects of LH are apparent within 2 h, but shortly thereafter the secretion is increased to give a surge in LH. In contrast, the decrease in FSH is not observed until 4–5 h after oestrogen injection and continues for up to 24 h. The evidence available (Clarke, 1987) supports the view that this short-term negative effect is the result of a pituitary action of oestrogen and does not involve effects on GnRH secretion.

The long-term negative feedback effects are seen when oestradiol alone holds LH concentrations below those of untreated ovariectomized ewes (Diekman & Malven, 1973). Oestradiol in combination with progesterone in the correct sequence and doses can result in changes in LH concentration in ovariectomized ewes which mimic those of intact, cyclic animals (Goodman *et al.*, 1981), and the effects of these steroids are exerted via GnRH secretion (Clarke, 1987).

The situation with FSH is different. Although oestradiol has a long-term negative influence on FSH secretion, none of the steroid replacements maintained FSH concentrations in the physiological range, although some suppression was evident (Goodman *et al.*, 1981). Furthermore, the rise in oestradiol in the preovulatory period could not account for the low basal FSH concentrations observed in that period (Goodman *et al.*, 1981), and an effect of steroids could not account for the selective increase in FSH on Day 1 of the cycle. These results supported the hypothesis that an ovarian hormone other than oestradiol and progesterone inhibits FSH secretion, and that this putative hormone is selective for FSH because the secretion of LH can be accounted for by the combination of oestradiol and progesterone in the female. The remainder of this review will consider the evidence that the hormone is the gonadal peptide, inhibin.

Control of FSH secretion by inhibin

Inhibin is defined as a gonadal peptide which selectively suppresses the secretion of FSH. Although inhibin was discovered as a testicular hormone (see Baker *et al.*, 1982), it is also present in the ovary (Baker *et al.*, 1982) particularly in follicular fluid (cow: de Jong & Sharpe, 1976; sheep: Tsonis *et al.*, 1983) which has proved a useful source of inhibin activity for experimentation and purification of the hormone. In males, the activity is found in testicular extracts, rete testis fluid, testicular lymph, seminal plasma and in media from cultures of seminiferous tubules and Sertoli cells (Baker *et al.*, 1982).

The gonadal fluids and extracts containing inhibin activity have been shown to suppress FSH secretion selectively or preferentially in in-vivo and in-vitro test systems (Baker *et al.*, 1982). FSH secretion is suppressed by follicular fluid in ovariectomized (Cummins *et al.*, 1983; Findlay *et al.*, 1985) and intact (Miller *et al.*, 1982) ewes and castrated males (Findlay *et al.*, 1985), and by rete testis fluid in castrated rams (Hudson *et al.*, 1979). Bovine follicular fluid will also selectively suppress the plasma concentrations of FSH in cows (Quirk & Fortune, 1986). In many of these experiments the effects of inhibin activity *in vivo* have been dose dependent. Unlike oestradiol negative feedback (Goodman & Karsch, 1981), there does not appear to be an influence of season on the ability of inhibin to suppress FSH secretion (Findlay *et al.*, 1985). Both ovine follicular fluid

(Tsonis *et al.*, 1983, 1986) and rete testis fluid (Hudson *et al.*, 1979; Tsonis *et al.*, 1986) selectively suppress FSH in rat and sheep pituitary cell assays *in vitro*. The in-vivo post-castration suppression of FSH and the in-vitro pituitary cell culture assays are accepted as being the most specific and reliable assays at present available for inhibin (Hudson *et al.*, 1979; Baker *et al.*, 1982).

Isolation and characterization of inhibin

Inhibin has been a difficult molecule to isolate and purify and so there has been considerable confusion about its physico-chemical characteristics (see de Jong & Robertson, 1985; Findlay, 1986). This is due to a combination of factors including the source of inhibin, ill-defined bioassay systems, the lack of standard reference preparations, the nature of the molecule itself and the inability of standard purification procedures to separate inhibin. Recent reports of the purification of inhibin over 3000-fold from cow (Robertson *et al.*, 1985, 1986; Fukuda *et al.*, 1986), sheep (Leversha *et al.*, 1986) and pig (Miyamoto *et al.*, 1985; Ling *et al.*, 1985; Rivier *et al.*, 1985) follicular fluid have resulted in a convergence of opinion about the physico-chemical properties of inhibin in the ovary. Inhibin has an apparent molecular weight ranging from 31 000 to 100 000 of which the forms of M_r 31–32 000 and 55 000–65 000 have been more extensively purified. Two forms of bovine inhibin have been purified: one is a protein of M_r 58 000 which dissociated into subunits of 43 000 (α) and 15 000 (β) (Robertson *et al.*, 1985), and the other is a protein of M_r 31 000 consisting of α (M_r 20 000) and β (M_r 13–15 000) subunits (Robertson *et al.*, 1986; Fukuda *et al.*, 1986). It was shown that the form of M_r 31 000 could be generated by including an acid pH precipitation step in the purification procedure for cow follicular fluid (Robertson *et al.*, 1986), suggesting that cleavage of the α-subunit resulted in the smaller form. This was confirmed by cloning and sequence analysis of cDNA species coding for the 2 subunits of bovine inhibin (Forage *et al.*, 1986); the α subunit contained a cleavage site at residues 165, 166 which would produce the form of M_r 20 000 (αc fragment) from that of M_r 43 000 (Fig. 1).

Ovine inhibin has been isolated as forms of M_r 67 000 and 32 000, the latter being purified to homogeneity and consisting of α (M_r 20–21 000) and β (M_r 16 000) subunits (Leversha *et al.*, 1986). Similarly, porcine inhibin of M_r 32 000 has been purified and consists of α (M_r 20 000) and β (M_r 13 000) subunits (Miyamoto *et al.*, 1985; Ling *et al.*, 1985; Rivier *et al.*, 1985). Ling *et al.* (1985) reported 2 forms of porcine inhibin (A and B) based on heterogeneity of the amino acid sequence in the β subunit, which was subsequently confirmed by cloning and sequence analysis of cDNA (Mason *et al.*, 1985). This heterogeneity has not been observed with bovine or ovine inhibin. In all species examined, potential glycosylation sites appear to be confined to the α-subunit.

The results suggest that at least 3 genes are present in the ovary which code for the subunits of

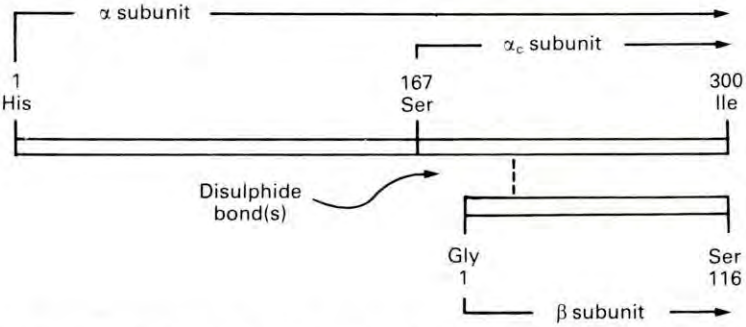

Fig. 1. Subunit structure of bovine inhibin based on sequence analysis of complementary DNA coding for the two subunits. DNA base sequence numbers are given at the beginning and end of each line and at base number 167 in the α subunit, which is a potential cleavage site. (After Forage *et al.*, 1986.)

inhibin, each of which share considerable sequence homology, indicative of a common ancestral gene (Mason *et al.*, 1985). Cloning and sequencing of cDNA coding for human inhibin (Mason *et al.*, 1986) have confirmed the similarity of the genes coding for inhibin amongst different species and underlined the extensive homology of the subunits between species. Interestingly, it is the β-subunit of inhibin which shares sequence homology with transforming growth factor-β (Derynck *et al.*, 1985) and Müllerian inhibiting substance (Cate *et al.*, 1986), raising the possibility that these are members of a family of proteins which influence cell function in similar manner. It is the β-subunits of inhibin which form homo- (Vale *et al.*, 1986) and hetero- (Ling *et al.*, 1986) dimers and are found in pig follicular fluid which have potent FSH stimulating activity *in vitro*. Although these β-dimers may be secreted by the gonad and act on the pituitary (see above), they may also have a local, intragonadal action. The fact that TGF-β enhanced and porcine inhibin of M_r 32 000 antagonized the ability of FSH to stimulate secretion of oestradiol by rat granulosa cells *in vitro* (Ying *et al.*, 1986b) underlines the possibility of a paracrine autocrine action of these inhibin-related polypeptides.

Published data on purification of inhibin from the male is not as advanced as that from ovarian follicular sources (see de Jong & Robertson, 1985; Findlay, 1986). Sairam *et al.* (1981) reported an inhibin of M_r 18 000 in bovine seminal plasma to be purified 500-fold. Activity of an M_r 20 000 protein has been isolated from ovine testicular extracts and claimed to be purified 770-fold (Moudgal *et al.*, 1985), but the assay used to monitor activity has been questioned (Hudson *et al.*, 1979).

The availability of pure bovine inhibin has allowed development of a radioimmunoassay which is applicable to cattle and humans (McLachlan *et al.*, 1986) and should ensure more rapid progress in our knowledge of the physico-chemical characteristics and physiology of inhibin. However, in view of the existence of dimers based on the β-subunits of inhibin (Vale *et al.*, 1986; Ling *et al.*, 1986) and their homology with TGF-β and Müllerian inhibiting substance (see above), it will be important to establish the specificity of the antibodies raised against inhibin. The antiserum to bovine inhibin (M_r 58 000) cross-reacts with the form of M_r 32 000 but not with the subunits (McLachlan *et al.*, 1986), suggesting that it is directed against conformational sites on the intact molecule and is therefore specific for inhibin.

Production and transport of inhibin

Inhibin activity is produced by the granulosa cells of the ovary (cow: Henderson & Franchimont, 1981) and the Sertoli cells of the testis (see Baker *et al.*, 1982). The inhibin content, and to a lesser extent the concentration, increases with follicular size and decreases with atresia of large follicles in sheep (Tsonis *et al.*, 1983) and cows (Henderson *et al.*, 1984). Treatment of hypophysectomized ewes with PMSG increased the inhibin content of the ovary (Cahill *et al.*, 1985). However, treatment of bovine granulosa with gonadotrophin *in vitro* did not significantly alter inhibin production, although micromolar doses of androgen did cause a stimulation (Henderson & Franchimont, 1983).

The route by which inhibin leaves the testis to reach the peripheral circulation is unclear (see Baker *et al.*, 1982). It might pass through the seminiferous tubules and rete testis and be absorbed into the circulation at the head of the epididymis. It is also likely to enter the testicular capillaries from the lymph spaces surrounding the seminiferous tubules. Baker *et al.* (1982) have estimated that the daily output of inhibin in testicular lymph is at least equal to that in rete testis fluid of the ram and sufficient to maintain a tonic inhibition of FSH. They were unable to measure inhibin activity in testicular vein blood of rams, using the rat pituitary cell bioassay.

Using a similar bioassay, Findlay *et al.* (1986) measured inhibin in ovarian lymph of PMSG-treated ewes, but could not detect it in ovarian or jugular venous plasma. Destruction of visible follicles by electrocautery was followed by a rapid decline in secretion of inhibin in ovarian lymph. Findlay *et al.* (1986) concluded that inhibin could reach the peripheral circulation by the

ovarian lymphatic system, particularly in the luteal phase when lymph flow rates are high. However, because venous outflow from the gonads is many times greater than lymph flow, relatively small concentrations of inhibin in venous plasma can make a major contribution to peripheral concentrations. Tsonis *et al.* (1986) have developed a more sensitive in-vitro assay using sheep pituitary cells which detected inhibin activity in ovarian venous plasma of a control and an FSH-treated ewe, and in peripheral plasma of the FSH-treated ewe. They showed higher concentrations of inhibin in the ovarian vein than in peripheral blood, particularly after treatment with FSH.

The half-life of ovine inhibin activity in peripheral blood of ovariectomized ewes was calculated to be around 45–50 min (Findlay *et al.*, 1986). In intact ewes, Tsonis *et al.* (1986) observed 2 half-life components of 18–24 and 50–60 min after injection of ovine follicular fluid. In both studies, the inhibin concentrations in plasma remained elevated after injection for up to 30 min before exponentially decaying, and in the intact ewes duration of the plateau was dose-dependent. This may reflect equilibration of inhibin to a carrier protein, dissociation of bound or polymeric forms, or saturation of a cleavage enzyme necessary for clearance or release from a polymeric form. It could also reflect the presence of the FSH-stimulating β-dimers (Vale *et al.*, 1986; Ling *et al.*, 1986) in ovine follicular fluid which would reduce the 'apparent' inhibitory effect of inhibin in the in-vitro bioassay until such time as they are cleared from the circulation. We suggest that the β-dimers would have a more rapid clearance than inhibin because of the lack of glycosylation sites on the β-subunits (see above).

Action of inhibin

That inhibin has a pituitary site of action is amply demonstrated by the effect of extracts and fluids containing inhibin activity (Baker *et al.*, 1982) and pure inhibins from cows, pigs and sheep (Robertson *et al.*, 1985, 1986; Miyamoto *et al.*, 1985; Rivier *et al.*, 1985; Ling *et al.*, 1985; Fukuda *et al.*, 1986; Leversha *et al.*, 1986) on FSH release by pituitary cell cultures *in vitro*. The effect of pure inhibin (forms of $M_r \sim 58\,000$ and $\sim 32\,000$) at lower doses is specific for FSH release in that there were no significant changes in LH or prolactin release *in vitro*. At higher doses, pure inhibin will suppress LH *in vitro* (Farnworth *et al.*, 1986). A direct pituitary site of action was recently demonstrated *in vivo* (Clarke *et al.*, 1986) when it was shown that treatment with ovine follicular fluid caused a 95% reduction in plasma FSH concentrations in ovariectomized ewes that had been subjected to hypothalamo–pituitary disconnection and in which gonadotrophin secretion had been reinstated with 250 ng pulses of GnRH every 2 h.

Whether or not inhibin has a hypothalamic action to limit secretion of GnRH is less certain. Intrahypothalamic and intraventricular injections of inhibin activity suppressed plasma FSH concentrations in laboratory rodents (see Clarke *et al.*, 1986, for references) but the mechanisms for these effects were not clear.

High doses of inhibin activity are known to inhibit both FSH and LH release induced by endogenous GnRH in ovariectomized ewes (Findlay *et al.*, 1985). Since LH pulses reflect GnRH secretion (Clarke & Cummins, 1982) it is possible to assess, indirectly, the effects of high doses of inhibin on hypothalamic function by examining LH pulse frequency in ovariectomized ewes. Similar studies in intact ewes (McNeilly, 1984; Wallace & McNeilly, 1985) are difficult to interpret because of confounding effects of oestrogens on GnRH pulse frequency (Clarke, 1987). Large doses of ovine follicular fluid given to ovariectomized ewes causes a significant reduction in plasma FSH and LH concentration, the latter probably due to a reduction in LH pulse amplitude in 3/4 animals, but no change in LH pulse frequency (Clarke *et al.*, 1986). The ability of follicular fluid containing inhibin activity to reduce LH pulse amplitude by a direct action on the pituitary was confirmed in experiments using ovariectomized ewes with hypothalamo–pituitary disconnection (Clarke *et al.*, 1986). Overall, these experiments suggest that inhibin does not act on hypothalamic or extrahypothalamic centres that determine GnRH pulse frequency. The major effect of inhibin is to

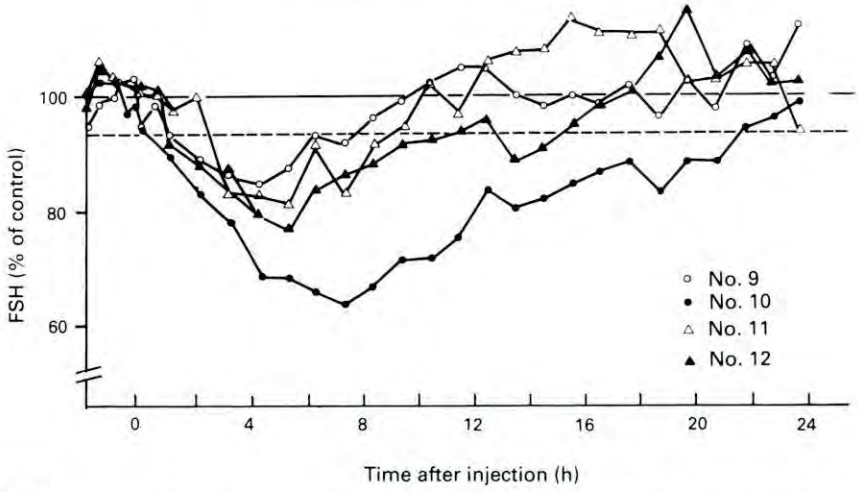

Fig. 2. Time course of suppression of plasma FSH in 4 ovariectomized ewes after intracarotid injection of 470 units of pure bovine inhibin (M_r 31 000). The broken line represents the range of FSH in plasma of 4 control ewes which received 2 ml serum from an hypophysectomized ewe. (Unpublished observations of J. K. Findlay, I. J. Clarke & D. M. Robertson.)

reduce FSH secretion by an action on the pituitary gland and there are components in follicular fluid, including inhibin, which can affect LH pulse amplitude, probably by a direct pituitary action.

It is possible to attribute the FSH-suppressing activity in follicular fluid to inhibin in the experiments described above because of the in-vitro actions of the purified inhibins (see above) and the recent demonstration that pure bovine inhibin (M_r 31 000) is also active *in vivo* (J. K. Findlay, I. J. Clarke & D. M. Robertson, unpublished observations). Intracarotid injection of pure inhibin into ovariectomized ewes resulted in a suppression of plasma FSH concentrations (Fig. 2) with a time course similar to that produced by bovine follicular fluid (J. K. Findlay & I. J. Clarke, unpublished), and with no significant effect on plasma LH. Unlike the responses of intact ewes (Miller *et al.*, 1982; Wallace & McNeilly, 1985) and cows (Quirk & Fortune, 1986), there was no rebound effect on plasma FSH in ovariectomized ewes after injection with bovine follicular fluid (J. K. Findlay & I. J. Clarke, unpublished) or pure bovine inhibin. This indicates that the rebound effect is associated with the gonads, presumably via a reduction in the endogenous secretion of oestradiol and inhibin as a result of decreasing gonadotrophin stimulation. There could also have been a direct effect of follicular fluid (Cahill *et al.*, 1985) and inhibin (Ying *et al.*, 1986b) on ovarian function in the intact ewes, to reduce gonadal feedback.

Repeated treatment of intact sheep with bovine follicular fluid resulted in a gradual onset of refractoriness of the FSH-suppressing activity (Wallace & McNeilly, 1985; Henderson *et al.*, 1986). This phenomenon is unlikely to be due to an immune response to bovine follicular fluid, given the relatively short duration of treatment (Wallace & McNeilly, 1985). It may be due to a long-term reduction in ovarian production of inhibin and oestradiol allowing a partial increase in pituitary FSH secretion (Henderson *et al.*, 1986). Or there could be a refractory (down-regulation?) effect at the pituitary level.

Inhibin and fecundity

FSH controls gamete production in the gonads and therefore manipulation of FSH secretion through an influence on inhibin production and action offers a means of improving fecundity in domestic animals.

One way to achieve this would be to select animals with low inhibin production. The highly fecund Booroola Merino ewe has a lower ovarian content of inhibin than control Merinos (Cummins *et al.*, 1983). This would lead to higher FSH secretion and the consequent effects on the gonads and suggests variation in the inhibin gene may be the basis of the increased fecundity in the Booroolas (Bindon *et al.*, 1985b). D'man sheep are also highly fecund and have high circulatory FSH concentrations (Lahlou-Kassi *et al.*, 1984) but their inhibin levels have not been reported.

A second method to increase fecundity would be to neutralize circulating inhibin by active or passive immunization. Cummins *et al.* (1986) observed an increase in ovulation rate after immunization of ewes with a fraction of bovine follicular fluid containing inhibin activity. The plasma FSH concentrations in the immunized animals were increased compared with controls (Al-Obaidi *et al.*, 1987), and plasma from immunized ewes inhibited the FSH-suppressing activity of bovine follicular fluid administered to ovariectomized ewes (Al-Obaidi *et al.*, 1986), suggesting that antibodies had been raised against inhibin. Now that inhibin has been purified and the cDNA cloned (see above) it should be possible to repeat these experiments with pure inhibin, its subunits and fragments to assess whether this is a practical method of improving fecundity.

Finally, it has been observed (Miller *et al.*, 1982; Wallace & McNeilly, 1985; Henderson *et al.*, 1986; Quirk & Fortune, 1986) that, 24–36 h after cessation of treatment of intact ewes and cows with follicular fluid, there is a rebound effect on plasma FSH such that concentrations exceed those in control animals. This treatment increased the proportion of multiple ovulating ewes (Wallace & McNeilly, 1985; Henderson *et al.*, 1986), an effect attributed to the increase in FSH. If similar increases in ovulation rate can be achieved using pure or synthesized inhibin during the late luteal phase of the cycle, this method may also improve fecundity of animals.

Conclusions

The secretion of FSH and LH has been shown to vary independently in physiological and experimental situations, indicating independent regulatory mechanisms for each gonadotrophin. The regulation of FSH can occur at three levels, i.e. hypothalamus and higher brain centres, the pituitary gland and the gonad. At the hypothalamus, specific FSH-releasing hormones have been postulated but never proven conclusively. The demonstration that homo- and heterodimers of the β-chain of inhibin have potent FSH-releasing activities offers new scope in that area. At the pituitary, there is evidence that the gonadotrophs which synthesize and secrete FSH have a much greater capacity to continue that function than those which produce LH. This indicates intra- and intercellular differences in the regulation of LH and FSH and offers an experimental challenge in the area of control of synthesis, processing and packaging of the gonadotrophin subunits. Finally, regulation of FSH occurs at the level of the gonad through production of agents such as inhibin which selectively influence FSH secretion.

Inhibin has now been isolated from follicular fluid of cows, sheep and pigs and purified to homogeneity. The cDNA coding for bovine, porcine and human inhibin messenger RNA has been cloned and sequenced. Inhibin is a heterodimer in which a larger, glycosylated α-chain (M_r 20 000–43 000) is joined to a smaller β-chain (M_r 13–15 000) by disulphide bridges. Pure inhibin is biologically active *in vivo* and *in vitro* and radioimmunoassays for measuring inhibin have been developed. Collectively, this should provide an impetus to describe the physiology of inhibin in the next few years.

There are already some surprises. Inhibin shares amino acid sequence homology with the cell regulator, TGF-β, and with Müllerian inhibiting substance, suggesting that these peptides belong to a gene family whose products have cell regulatory properties at endocrine, paracrine and/or autocrine levels. In the case of inhibin, reassembly of its subunits to form β-dimers can reverse the activity from inhibitory to stimulatory, indicating that the point of assembly of the subunits is a key control point for subsequent biological activity. Manipulation of inhibin and its β-chain dimers by

analogue treatment, immunization or genetic selection and engineering will provide new insights into the control of reproduction and may offer a means of regulating fecundity of domestic animals.

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