Variation in pituitary expression of mRNAs encoding the putative inhibin co-receptor (betaglycan) and type-I and type-II activin receptors during the chicken ovulatory cycle

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Abstract

Secretion of LH and FSH from the anterior pituitary is regulated primarily by hypothalamic GnRH and ovarian steroid hormones. More recent evidence indicates regulatory roles for certain members of the transforming growth factor β (TGFβ) superfamily including inhibin and activin. The aim of this study was to identify expression of mRNAs encoding key receptors and ligands of the inhibin/activin system in the hen pituitary gland and to monitor their expression throughout the 24–25-h ovulatory cycle. Hens maintained on long days (16 h light/8 h dark) were killed 20, 12, 6 and 2 h before predicted ovulation of a midsequence egg (n=8 per group). Anterior pituitary glands were removed, RNA extracted and cDNA synthesized. Plasma concentrations of LH, FSH, progesterone and inhibin A were measured. Real-time quantitative PCR was used to quantify pituitary expression of mRNAs encoding betaglycan, activin receptor (ActR) subtypes (type I, IIA), GnRH receptor (GnRH-R), LH β subunit, FSH β subunit and GAPDH. Levels of mRNA for inhibin/activin βA and βB subunits, inhibin α subunit, follistatin and ActRIIB mRNA in pituitary were undetectable by quantitative PCR (<2 amol/reaction). Significant changes in expression (P<0.05) of ActRIIA and betaglycan mRNA were found, both peaking 6 h before ovulation just prior to the preovulatory LH surge and reaching a nadir 2 h before ovulation, just after the LH surge. There were no significant changes in expression of ActRI mRNA throughout the cycle although values were correlated with mRNA levels for both ActRIIA (r=0.77; P<0.001) and betaglycan (r=0.45; P<0.01). Expression of GnRH-R mRNA was lowest 20 h before ovulation and highest (P<0.05) 6 h before ovulation; values were weakly correlated with betaglycan (r=0.33; P=0.06) and ActRIIA (r=0.34; P=0.06) mRNA levels. Expression of mRNAs encoding LH β and FSH β subunit were both lowest (P<0.05) after the LH surge, 2 h before ovulation. These results are consistent with an endocrine, but not a local intrapituitary, role of inhibin-related proteins in modulating gonadotroph function during the ovulatory cycle of the hen, potentially through interaction with betaglycan and ActRIIA. In contrast to mammals, intrapituitary expression of inhibin/activin subunits and follistatin appears to be extremely low or absent in the domestic fowl.

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Introduction

The regulation of anterior pituitary function is achieved through the balance of central, local and endocrine signals. Gonadotroph cells of the anterior pituitary gland produce both luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which play fundamental roles in the control of ovarian function in the domestic hen. Pituitary gonadotrophin secretion is regulated by the hypothalamic peptide gonadotrophin-releasing hormone (GnRH; also known as LHRH-I; King et al. 1989), acting through GnRH receptors (GnRH-Rs; Millar & King 1983, Kawashima et al. 1992a). Ovarian steroid-feedback mechanisms also modulate gonadotrophin secretion through both direct and indirect (GnRH-mediated) actions (Luck & Scanes 1980, King et al. 1989, Kawashima et al. 1992b).

The transforming growth factor β (TGFβ)-superfamily members inhibin and activin are dimeric glycoproteins first identified in gonadal fluids of pigs and cows for their ability to suppress (inhibin) or stimulate (activin) pituitary FSH secretion (de Jong 1988, Ying 1988). The ovary is a major source of inhibin and activin and both molecules have also been implicated as local autocrine/paracrine regulators of folliculogenesis in avian and mammalian species (Findlay 1993, Lovell et al. 2001, 2002a, 2002b, Knight & Glister 2003). Moreover, in mammals the anterior pituitary is a site of inhibin/activin subunit and follistatin (activin-binding protein) expression and substantive evidence indicates autocrine/paracrine roles for
these proteins in the regulation of gonadotroph function (see a review by Gregory & Kaiser 2004). However, the extent to which ligands and receptors of the inhibin–activin–follistatin system are expressed in the avian pituitary gland has not been investigated.

Two forms of inhibin exist, termed inhibin-A and -B; these consist of a common α subunit linked through a disulphide bond to one of two alternative β subunits, termed βA and βB, respectively. There are three forms of activin, termed activin A, B and AB, which are disulphide-linked dimers of two inhibin β subunits (βAβA, βBβB and βAβB respectively). Activins signal through interaction with serine/threonine kinase receptors on the cell surface (Ethier & Findlay 2001). There are two main types of activin receptor, ActRI and ActRII, the latter of which determines ligand-binding specificity. Two ActRII subtypes (A, B) have been cloned in mammalian (Donaldson et al. 1992, Ethier et al. 1994) and avian (Ohuchi et al. 1992, Stern et al. 1995) species and both have a high-affinity binding site for activin. Likewise, ActRI exists as two subtypes (A, B) in mammals (Attisano et al. 1993, Tsuchinda et al. 1995) but only one subtype has so far been identified in birds. Although activin does not bind directly to ActRI (Matsuzaki et al. 1993, ten Dijke et al. 1993), binding of activin to ActRII promotes the formation of a hetero-oligomeric complex of the two receptor types, leading to activation of the activin-induced signalling pathway (Attisano et al. 1993, ten Dijke et al. 1993, Tsuchinda et al. 1993, 1995).

The mechanism of inhibin signal transduction has not been fully elucidated but the favoured model involves inhibin interfering with activin-induced oligomerization of ActRI and ActRII (Gray et al. 2002). In this regard, both ActRII subtypes have been shown to bind inhibin, albeit with low affinity (Mathews and Vale 1991, Attisano et al. 1993). A non-signalling cell-surface molecule termed betaglycan (also known as TGFβ type-III receptor) also binds inhibin-A with high affinity. Moreover, binding to betaglycan increases the affinity of inhibin–A for ActRII, rendering inhibin-A more effective at blocking activin-induced oligomerization of ActRI and ActRII (Lewis et al. 2000, Matzuk 2000). Thus, betaglycan is considered to be an important co-receptor for inhibin.

The objectives of the present study were firstly to determine whether betaglycan, activin receptor subtypes (ActRI, IIA and IIB) and inhibin-related proteins (inhibin/activin α, βA and βB subunits and follistatin) are expressed in the hen pituitary gland and secondly to quantify mRNA expression levels throughout the hen ovulatory cycle in order gain an insight into potential endocrine and/or local intrapituitary roles of these proteins in the avian reproductive system. To help achieve this we also quantified expression of mRNAs for GnrH-R, LH β subunit and FSH β subunit in the same pituitary glands and plasma samples were analysed for LH, FSH, inhibin-A and progesterone.

Materials and Methods

Experimental animals

Laying hens (Goldline) towards the end of the first year of lay, with a clutch average of at least five eggs, were caged individually and maintained under a standard long-day photo-schedule of 16 h of light and 8 h of darkness, at an ambient temperature of 21–23 °C. Food and water were freely available. Ovipositions were recorded using time-lapse recording technology and used to predict the time of ovulation.

Recovery of pituitary glands, RNA purification and cDNA synthesis

Hens (n=8 per group) were killed by cervical dislocation 4, 12, 18 and 22 h after predicted ovulation of a mid-sequence egg. Pituitary glands were dissected, weighed and homogenized in a 15-times-volume of Tri-reagent (Sigma) using an Ultra-Turrax T8 homogenizer (IKA, Staufen, Germany). RNA was subsequently purified as described in the standard Tri-reagent protocol. The final RNA pellet was resuspended in 100 µl nuclease-free water (containing RNA Secure; Ambion), and then treated with RNase-free DNase (15 min at 37 °C; RQ1; Promega). The purified RNA was re-purified using a 15-times volume of Tri-reagent. The resultant purified RNA was resuspended in 50 µl nuclease-free water (containing RNA Secure).

RNA was then quantified on a spectrophotometer (GeneQuant; GE Healthcare). cDNA was synthesized using ImProm-II reverse transcriptase (using protocol and buffers provided; Promega), 1 µg RNA, 0·5 µg random hexamer primers (MWG-Biotech), dNTPs (0·5 mM final; Promega) and 0·5 µl RNase inhibitor (40 U/µl; Ambion). cDNA synthesis was terminated by heat-inactivation (15 min at 70 °C). cDNA samples were treated with 1 µl RNase cocktail (0·5 U/µl RNase A and 20 U/µl RNase T1; Ambion) and 0·5 µl RNase H (40 U/µl; Ambion), which specifically degrades the RNA in RNA–DNA hybrids. A 1 µl aliquot of cDNA was removed for estimation using a fluorometric assay (Oligreen ssDNA quantification assay; Molecular Probes).

Blood samples were collected prior to cervical dislocation into heparinized tubes, centrifuged at 3000 g for 20 min at 4 °C and the plasma was stored at ~20 °C until required.

Recovery of positive control ovarian tissue, RNA purification and cDNA synthesis

Hens (n=4) were killed by cervical dislocation 12 h after predicted ovulation of a mid-sequence egg. The F4–F6 follicles were dissected into granulosa and theca layers and combined as separate pools. Tissue pools were weighed,
extracted and cDNA was prepared as described above. The granulosa tissue from F4–F6 preovulatory follicles have previously been demonstrated to express mRNA for follistatin, inhibin/activin mRNA has been demonstrated to express mRNA for follistatin, inhibin/activin mRNA, and expression of mRNAs encoding receptors and gonadotrophin subunits varied at different time points. Levels of expression of each mRNA detected, including GAPDH mRNA, was normalized to total cDNA. GAPDH mRNA expression normalized in this way did not vary across the cycle. P<0.05 was considered to be significant. Unless otherwise stated, values are means ± s.e.m. (n=8). Ovulatory cycle data are expressed relative to the predicted time of ovulation.

### Results

**Plasma inhibin-A, LH, FSH and progesterone during the hen ovulatory cycle**

Plasma concentrations of inhibin-A, progesterone, LH and FSH were determined to verify the correct timing of pituitary collection relative to predicted time of ovulation. As shown in Fig. 1 the changes in plasma LH and progesterone observed were consistent with the well-established preovulatory peak in plasma LH and progesterone ~4 h before ovulation (Etches & Cunningham 1976). Likewise, as reported previously (Lovell et al. 2000), plasma inhibin-A also showed a significant (P<0.05) pattern of change throughout the ovulatory cycle, whereas FSH concentrations did not vary significantly.

**Pituitary expression of mRNA for betaglycan and activin receptor subtypes (types I, IIA and IIB) during the hen ovulatory cycle**

Changes in pituitary expression of mRNAs encoding betaglycan, ActRI and ActRIIA during the hen ovulatory cycle are shown in Fig. 2. Concentrations of ActRIIA mRNA peaked at 6 h prior to the expected time of ovulation (around the onset of LH surge) at 6-FAM, 6-carboxyfluorescein; TAMRA, carboxytetramethylrhodamine.

### Table 1 TaqMan primer and probe sequences used in this study

<table>
<thead>
<tr>
<th>Target</th>
<th>Forward primer (5’ to 3’)</th>
<th>Reverse primer (5’ to 3’)</th>
<th>TaqMan probe 5’ [6-FAM] to 3’ [TAMRA]</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>ActRI</td>
<td>cggaggtctctgacagaaaa</td>
<td>gccgatatgtcacccttcttg</td>
<td>catccagcagctgctctgccgcccttcttg</td>
<td>AJ318064</td>
</tr>
<tr>
<td>ActRIIA</td>
<td>tcpacagaaagtctttaattttg</td>
<td>cggccagatgctgtgctttcttg</td>
<td>tcttcaggctgtaactgcttgcccttcttg</td>
<td>D31899</td>
</tr>
<tr>
<td>ActRIIB</td>
<td>aactgcggcactcactgcttta</td>
<td>aacagaggctgctgtgctttcttg</td>
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</tr>
<tr>
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<td>agggcgctgtgctgtgctttcttg</td>
<td>tggcagcttcctgtgctctgcccttcttg</td>
<td>Noce et al. (1989)</td>
</tr>
<tr>
<td>GnRH-R</td>
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<td>actgagcaatgtgacacagcatgt</td>
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<tr>
<td>LH β subunit</td>
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<td>tggcagcttcctgtgctctgcccttcttg</td>
<td>U31223</td>
</tr>
<tr>
<td>FSH β subunit</td>
<td>aacaggtctctgacagaaaa</td>
<td>gacacaggtctgtgctttcttg</td>
<td>tggcagcttcctgtgctctgcccttcttg</td>
<td>L01121</td>
</tr>
<tr>
<td>Inhibin α subunit</td>
<td>aacaggtctctgacagaaaa</td>
<td>gacacaggtctgtgctttcttg</td>
<td>tggcagcttcctgtgctctgcccttcttg</td>
<td>L01121</td>
</tr>
<tr>
<td>Inhibin β subunit</td>
<td>aacaggtctctgacagaaaa</td>
<td>gacacaggtctgtgctttcttg</td>
<td>tggcagcttcctgtgctctgcccttcttg</td>
<td>L01121</td>
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<tr>
<td>Follistatin subunit</td>
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<td>tggcagcttcctgtgctctgcccttcttg</td>
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<td>tggcagcttcctgtgctctgcccttcttg</td>
<td>L01121</td>
</tr>
</tbody>
</table>

6-FAM, 6-carboxyfluorescein; TAMRA, carboxytetramethylrhodamine.
20·7 amol/µg cDNA. Levels fell significantly (P<0·05) to a post-LH surge nadir (9·3 amol/µg cDNA), approximately 2 h before ovulation. At 20 and 12 h before ovulation ActRIIA mRNA levels were not significantly different from those 2 h prior to ovulation. ActRI mRNA levels were positively correlated with ActRIIA mRNA levels (r=0·77; P<0·001) but the changes during the cycle did not reach statistical significance. Betaglycan mRNA expression, like ActRI and ActRIIA, was greatest before the onset of the preovulatory LH/progesterone surge, with levels falling significantly (P<0·05) from 6 to 2 h prior to ovulation (range, 1·0–0·67 amol/µg cDNA). Betaglycan mRNA levels were positively correlated with both ActRI (r=0·45; P<0·01) and ActRIIA (r=0·39; P<0·05) mRNA levels. ActRIIB expression was below the detection limit of the Q-PCR procedure (2 amol/reaction) at all stages of the ovulatory cycle.

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Pituitary expression of mRNA for betaglycan, ActRI and ActRIIA in the pituitary gland of laying hens at four time points during the ovulatory cycle. Values are means ± S.E.M. (n=8 per time point) and means without a common letter are significantly different (P<0·05; ANOVA and Fisher’s PLSD test). For comparison the shaded areas show typical hormone profiles (mean ± S.E.M.) in hens blood-sampled longitudinally throughout the ovulatory cycle (Lovell et al. 2000).

Pituitary expression of mRNA for GnRH-R, LH β subunit and FSH β subunit during the hen ovulatory cycle

Pituitary expression of mRNAs encoding GnRH-R, LH β subunit and FSH β subunit during the hen ovulatory cycle are shown in Fig. 3. Expression of GnRH-R mRNA was lowest 20 h before ovulation (8·5 amol/µg cDNA). Expression increased (P<0·05) from 20 to 12 h before ovulation to a maximal value some 3-fold higher at 6 h before ovulation (29·8 amol/µg cDNA), before tending to fall (37%; not significant) after the LH surge. Levels of mRNA for FSH β subunit and LH β subunit were highest at 20 and 12 h before expected ovulation and at their lowest values (P<0·05) after the preovulatory LH surge, some 2 h before expected ovulation. There was a significant correlation between mRNA levels for the two gonadotrophin subunits (r=0·45; P<0·01).

Pituitary expression of mRNAs for inhibin/activin α, βA and βB subunits and follistatin during the hen ovulatory cycle

Levels of pituitary expression of mRNAs encoding inhibin/activin α, βA and βB subunit and follistatin were consistently below the detection limit of the Q-PCR procedure (<2 amol/reaction) at all stages of the ovulatory cycle. Positive control samples from hen follicular granulosa and theca tissue processed and analysed in the same manner had readily measurable amounts of these mRNA transcripts (see Table 2).
Pituitary expression of GAPDH mRNA during the hen ovulatory cycle

There were no significant differences (P>0.05) in pituitary expression of GAPDH mRNA across the hen ovulatory cycle (range, 0.43 ± 0.4–0.50 ± 0.07 fmol/µg cDNA).

Discussion

Inhibin, activin and follistatin were initially identified in mammals as gonadally derived proteins capable of modulating pituitary FSH secretion (Ying 1988). Subsequently it was discovered that activin and follistatin are also expressed in the mammalian anterior pituitary where they act as local modulators of gonadotroph function, including regulation of GnRH-R and FSH β subunit expression (DePaolo et al. 1991, Marshall et al. 1997). The avian ovary, like its mammalian counterpart, expresses inhibin, activin and follistatin (Davis & Johnson 1998, Knight et al. 2005), but it has not been determined whether an endogenous pituitary inhibin–activin–follistatin system exists in birds. Likewise, there is no information on expression of ActRs in the avian pituitary, although one recent study (Sweeney & Johnson 2005) confirmed that the inhibin coreceptor betaglycan is expressed in hen anterior pituitary.

Here we used quantitative reverse transcription PCR (Taqman) to examine mRNA expression of ActR subtypes (types I, IIA and IIB), betaglycan and inhibin–related proteins (follistatin and inhibin/activin α, βA and βB subunits) in the hen anterior pituitary and to monitor their levels throughout the ovulatory cycle. For positive control purposes we also evaluated expression of all eight mRNA transcripts in pooled extracts of hen ovarian theca and granulosa. Pituitary expression of GnRH-R was also investigated as activin has been shown to increase pituitary GnRH-R expression in rats (Braden & Conn 1992) and thereby affect FSH and LH secretion (Liu et al. 1995). GnRH-R mRNA levels was highest prior to the onset of the LH surge, as also demonstrated in the ewe (Brooks et al. 1993). This also accords with a study on Japanese quail showing that pituitary responsiveness to chicken GnRH-I was greater before the preovulatory LH surge than at any other time of the cycle (Connolly & Callard 1988). We also monitored expression of LH β and FSH β subunits to help interpret our findings in relation to gonadotrophin synthesis and plasma gonadotrophin levels during the cycle. Pituitary expression of LH β and FSH β subunits were both lowest after the preovulatory LH/progesterone surge about 2 h before ovulation, presumably reflecting transient desensitization of gonadotrophs to the stimulatory effect of GnRH. The disparity between mRNA and plasma FSH profiles may due to the processes of translation, post-translational modification and dimerization between the α and specific β subunit to form FSH prior to secretion by the pituitary gland.

Our results showed readily detectable expression of mRNA for betaglycan, ActRI and ActRIIA, GnRH-R, LH β and FSH β in hen anterior pituitary, consistent with this tissue as being a target site for inhibin/activin action. Ligand-induced association of type I and type II ActRs is required to mediate the biological response to activin (Carcamo et al. 1994) and so the presence of ActRI and ActRIIA in hen pituitary (and ovary) is compatible with activin responsiveness. Inhibin signalling is believed to involve binding of inhibin to betaglycan which, in turn, enhances the affinity of inhibin for the type II activin receptor making it a more effective activin antagonist.

Table 2 RNA transcripts detected in the hen pituitary gland by TaqMan quantitative PCR; F4–F6 granulosa and theca tissue were analysed as positive controls

<table>
<thead>
<tr>
<th>RNA transcript</th>
<th>Pituitary gland</th>
<th>F4–F6 granulosa</th>
<th>F4–F6 theca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaglycan</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ActRI</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ActRIIA</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ActRIB</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inhibin α subunit</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inhibin βA subunit</td>
<td>–</td>
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<tr>
<td>Inhibin βB subunit</td>
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<td>Follistatin</td>
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<td>GnRH-R</td>
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</tr>
<tr>
<td>LH β subunit</td>
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</tr>
<tr>
<td>FSH β subunit</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

+, detectable; –, undetectable; nd, not determined.
these transcripts was readily detected in hen ovarian subunit, inhibin/activin \( \alpha \) subunit, inhibin/activin \( \beta A \) and \( \beta B \) subunit, follistatin or ActRIIB in hen pituitary tissue even though each of these transcripts was readily detected in hen ovarian tissue samples. Hecht et al. (2000) and Davis & Johnson (1998) were also unable to detect inhibin/activin \( \beta B \) and follistatin mRNA expression in the laying-hen pituitary by Northern blot hybridization; however, they were able to detect expression in all extragonadal tissues analysed using Southern blotting of PCR-amplified cDNA. Expression of inhibin/activin \( \beta B \) and follistatin mRNA has been well documented in mammalian anterior pituitary tissue; several studies have also detected lower levels of expression of inhibin \( \alpha \) and \( \beta A \) subunit mRNA (Meunier et al. 1988, Marshall et al. 1997, Dalkin et al. 1998). In addition, pituitary follistatin mRNA level varies markedly during the rat oestrous cycle (Halvorson et al. 1994); mRNAs for inhibin/activin subunits are relatively uniform across the cycle (Halvorson et al. 1994). Thus it appears that, unlike mammals, the avian anterior pituitary lacks the capacity for endogenous production of inhibins (A, B), activins (A, B, AB) or follistatin. Alternatively, the relevant mRNAs may be expressed at such low levels in the laying hen as to be undetectable by quantitative reverse transcription PCR. Either way, our findings imply that there is little or no autocrine/paracrine regulation of gonadotrophin production by endogenous (pituitary-derived) inhibin, activin or follistatin in the hen, in marked contrast to mammalian species examined so far. Since the present observations are confined to adult laying hens (\textit{Gallus domesticus}) it would be unwise to exclude the possibility that birds in other physiological states (e.g. sexually immature, ovariectomized), male birds or other avian species do not exhibit significant pituitary expression of inhibin-related proteins. In this regard, gonadectomy in rats results in enhanced pituitary expression of inhibin/activin subunit and follistatin mRNA (Marshall et al. 1997).

Regarding the changes we observed during the hen oestrous cycle, levels of ActRIIA and betaglycan mRNA were significantly higher before the preovulatory LH/progesterone surge (6 h before ovulation), as compared with after the LH/progesterone surge some 2 h before ovulation. While ActRI expression did not vary significantly across the oestrous cycle, values were positively correlated with ActRIIA and betaglycan mRNA levels. As mentioned above, ActRIIB expression was below the detection limit throughout. Differential pituitary expression of ActR subtypes has been reported in other (mammalian) species including sheep and rat. For instance, in the ewe pituitary gland ActRIA, ActRIB, ActRIIA, ActRIIB and betaglycan were all detectable throughout the ovulatory cycle (Fafioffe et al. 2004). Levels of ActRIA, ActRIB and ActRIIB mRNA were significantly higher before the preovulatory surge and during the secondary surge of FSH as compared with during the preovulatory surge and the luteal phase. Levels of ActRIIA and betaglycan mRNA did not vary throughout the sheep oestrous cycle. Likewise in the rat pituitary expression of betaglycan mRNA did not fluctuate across the rat oestrous cycle (Bernard & Woodruff 2001). However, a subsequent immunolocalization study revealed that the amount of betaglycan protein on the surface of gonadotrophs does vary, being highly correlated with the inverse changes in serum inhibin and FSH levels around the time of the secondary FSH surge (Chapman & Woodruff 2003). Interaction between circulating inhibin and local (pituitary-derived) activin-B probably plays a key role in the generation of the secondary FSH surge which occurs independently of LH release and initiates follicular recruitment during the rat oestrous cycle. The observation (Corrigan et al. 1991) that in vitro immunoneutralization of endogenous activin-B increased FSH secretion by isolated rat pituitary cells reinforces this concept. Also, mice lacking inhibin have elevated activin and FSH levels (Matzuk et al. 1992, 1994) while mice lacking activin receptor type II receptor have suppressed FSH levels (Matzuk et al. 1995). Although the present study did not attempt to study the anatomical distribution of mRNA transcripts or their corresponding protein products in hen pituitary, Sweeney & Johnson (2005) recently showed by immunohistochemistry that there is strong colocalisation of betaglycan with FSH-containing cells in the hen pituitary, again supporting a functional role for inhibin in FSH regulation.

As with activin, other ligands of the TGF\( \beta \) superfamily bind to and form a hetero-oligomeric complex with two types of cell-surface receptor, designated types I and II. However, not all types I and II receptor associations elicit a cellular response (Attisano et al. 1993, ten Dijke et al. 1994a, 1994b). Through interaction with betaglycan, inhibin could also disrupt the interaction of type II receptors with other members of the TGF\( \beta \) superfamily, such as the bone morphogenetic proteins (Cook et al. 2004). However, the significance of this required further evaluation. There also is the possibility that other members of the TGF\( \beta \) superfamily may play an important role in the regulation of pituitary gonadotroph function in the hen. For example, TGF\( \beta \) can modulate the association of inhibin with betaglycan, with TGF\( \beta 2 \) being a more effective competitor than TGF\( \beta 1 \) for binding of inhibin--A to L\( \beta \)T2 cells (Ethier et al. 2002). Indeed, it has been shown that TGF\( \beta \) can rescue the activin A-induced promoter activity when inhibin--A is present. The physiological significance of this finding in the hen pituitary is as-yet unknown, but a similar role could occur as TGF\( \beta \) expression has been demonstrated in the chicken anterior pituitary (Chowdhury et al. 2003).
During ovarian preovulatory follicle development in the laying hen, inhibin-A is produced selectively by granulosa cells of the largest follicle (F1) of the preovulatory hierarchy (Lovell et al. 1998, 2000, 2001, 2003). Correspondingly, plasma inhibin-A levels vary in a cyclic manner during the hen ovulatory cycle (Lovell et al. 2000). Despite this, plasma FSH concentrations (Lovell et al. 2000) and pituitary FSH β subunit expression (this study) do not vary significantly during the ovulatory cycle. This contrasts with the dynamic changes in pituitary FSH secretion observed during the oestrous cycle in mammals and suggests a different functional relationship between inhibin-related proteins and FSH in chickens compared with mammals. Avian ovarian cycles do not have the equivalent of a luteal phase, as follicles are developing in what could be termed ‘constant follicular phase’, with hens ovulating and laying an egg approximately every 24–25 h. In the laying hen regular recruitment of a ‘selected’ ovarian follicle from the pool of pre–hierarchical follicles occurs despite a uniform level of circulating FSH. Variability in FSH–responsiveness is thought to explain how a single follicle is recruited to the preovulatory hierarchy each cycle (Tilly et al. 1991, Johnson et al. 2004, Knight et al. 2005). This is in contrast to mammals where interplay between endogenous pituitary activin and follistatin, and ovary-derived inhibin, acutely regulates FSH β subunit expression leading to transient peaks in plasma FSH and the FSH–dependent recruitment of a cohort of follicles, one or more of which may subsequently be selected for ovulation (Besecke et al. 1997, Gajewska et al. 2002, Webb et al. 2003).

In conclusion, the present study has shown that the putative inhibin co-receptor betaglycan and type I and IIA ActRs are expressed in the anterior pituitary gland of the domestic hen consistent with this tissue being a target of inhibin/activin action. The apparent absence of endogenous pituitary expression of inhibin/activin α, βA and βB subunits and follistatin in the hen stands in marked contrast to mammalian species in which an intrapituitary activin–follistatin system plays a prominent role in the regulation of gonadotroph function.

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