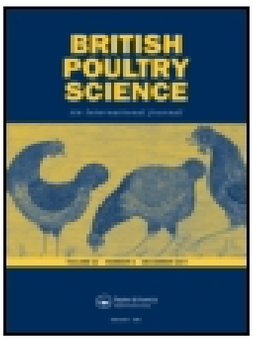

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# Immunocytochemical evidence for the existence of a lamprey luteinizing hormone-releasing hormone type III-like peptide in the chicken hypothalamus

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of LH was longer than between subsequent ovulatory surges. Not all ovulatory surges of LH led to laid eggs; of the 87 ovulatory surges of LH observed in the trial, 72 led to laid eggs. All surges of progesterone were associated with ovulatory surges of LH (24 of the 24 LH surges examined).

These data suggest that in egg-line hens, unlike some mammals, the first preovulatory LH surge is associated with the release of an oocyte, and that no phantom LH surges are associated with the onset of egg production.

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# Immunocytochemical evidence for the existence of a lamprey luteinizing hormone-releasing hormone type III-like peptide in the chicken hypothalamus

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Chicken luteinizing hormone-releasing hormone type I (cLHRH-I) was isolated from the hypothalamus of the chicken by King and Millar (1982). This decapeptide differed in only one amino acid from mammalian LHRH. Two years later, Myamoto *et al.* (1984) isolated cLHRH-II, which differed in 3 residues from cLHRH-I, namely at positions 5,7 and 8. The cellular distribution patterns of cLHRH-I and -II in the brain appeared to be quite different from one another, the diffuse localization of cLHRH-II at first glance looking much more like the distribution of a neuromodulator or a neurotransmitter substance than that of a releasing factor. Morphological data strongly suggested cLHRH-I as the major gonadotropin-releasing factor. Indeed, ample data are available showing the capacity of cLHRH-I to release both LH and FSH as a result of its direct action on the gonadotropes of the pituitary pars distalis. However, the mechanism behind the differential regulation of LH and FSH secretion in birds remains largely unknown.

In the rat, by contrast, McCann and co-workers have gathered substantial evidence for the separate hypothalamic control of FSH release, distinct from that of LH. These researchers showed that lamprey LHRH-III (lLHRH-III) has a potent, dose-related FSH- but not LH-releasing action in the rat (McCann *et al.*, 1998), suggesting that this peptide might be the long sought-after m-FSHRF. As a first stage in testing this hypothesis in the chicken, the present study reports immunocytochemical staining experiments on the chicken brain, in order to investigate the potential presence of a lamprey LHRH-III-like peptide in birds.

In order to increase the peptide content in the perikarya of the putative lamprey LHRH-III-producing neurons by blockage of the axonal peptide transport, 4-week-old broiler chickens were injected i.c.v. with 10 μg colchicin/100 g body weight. Twenty-four hours later, the birds were euthanized and immediately perfused with saline solution (containing 1% (w/v) $NaNO_2$ and 0·1% (w/v) heparin) and finally with Bouin Hollande Sublimate (BHS). Transverse 7 μm paraffin sections were prepared. Upon routine dewaxing and rehydration, the sections were pre-incubated for 1 h with 3% (v/v) normal goat serum. The primary antiserum, a polyclonal rabbit anti-serum towards lamprey LHRH-III (Sower *et al.*, 1995), was applied at a dilution of 1/1000 and left for incubation at 4°C for 48 h. Secondary reagents (from the Vectastain kit by Vector) were used as recommended by the manufacturer. Biotinylated goat anti-rabbit Ig and the preformed avidin–biotin–peroxidase complex, respectively, were applied for 30 min. Final detection of the peroxidase enzyme was performed using the glucose oxidase–DAB–nickel staining method as described by Shu *et al.* (1988).

In colchicin-treated birds, intensely stained neuronal cell bodies were observed throughout the paraventricular nucleus of the hypothalamus and also in more dorsolateral positions, approaching the lateral forebrain bundle and the dorsolateral nucleus of the anterior thalamus. In control animals, by contrast, immunopositive perikarya were hardly detectable (results not shown). Caudal projections of the above neurons were observed as a broad

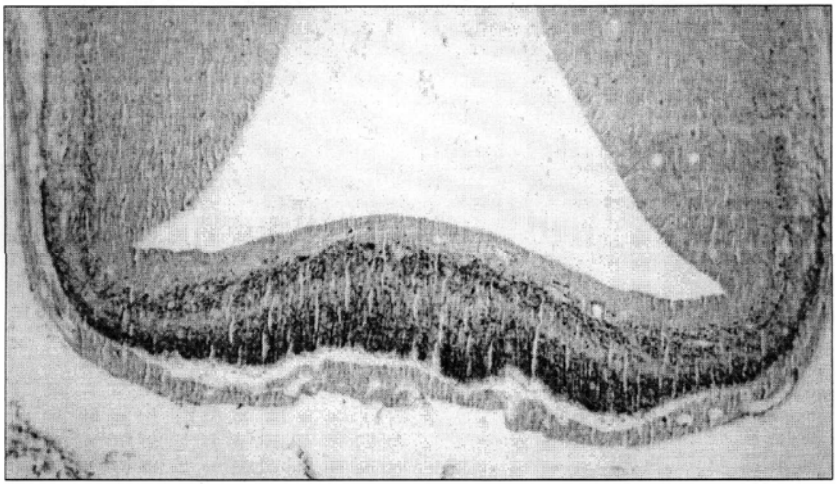

**Figure.** *Lamprey LHRH-III immunopositive fibers in the m*edian eminence of the chicken.



fiber tract running just dorsally of the dorsal supraoptic decussatio. Its complete trajectory could be followed until it reached the median eminence. Very intensely stained nerve fibers were easily detectable in both colchicin-treated and control animals, especially as they reached the median eminence (Figure). Additional immunopositive fibers were scarce throughout the brain.

The observed distribution, with neuronal cell bodies in the paraventricular nucleus of the hypothalamus and projections towards the median eminence, strongly suggests that lLHRH-III might indeed be a hypothalamic hypophysiotropic releasing factor in the chicken. However, much more research will be needed in order to conclusively prove that lLHRH-III is the missing specific chicken FSHRF, as has been suggested in the rat (Yu *et al.*, 1997). For this hypothesis to be true, it needs to be shown that the peptide is released into the hypophyseal portal blood system, that FSH cells express a specific receptor for the peptide and that FSH is released upon binding of lLHRH-III to its receptor.

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# Body weight, fat content, liver weight and plasma leptin concentrations in broiler breeder females reared under *ad libitum* feeding, restricted feeding or combinations of both until age of first egg

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Several studies in mammals have shown a relationship between obesity, plasma leptin concentrations and reproduction (Cunningham *et al.*, 1999; Foster and Nagatani, 1999). Similar studies in birds are lacking even though obesity is a common occurrence in poultry especially in broiler breeders under *ad libitum* feeding where reproductive rate is seriously hampered. The amount of feed given to