Autoregulation of central and peripheral growth hormone receptor mRNA in domestic fowl

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Abstract

Growth hormone (GH) regulates numerous cellular functions in many different tissues. A common receptor is believed to mediate these tissue-specific effects, suggesting that post-receptor signalling molecules or tissue sensitivity to GH may differ between tissues. Tissue sensitivity depends upon the abundance of GH receptors (GHRs), thus tissue-specific GHR regulation could enable tissue-specific GH actions. The comparative autoregulation of GHR gene transcription in central (whole brain or hypothalami) and peripheral (liver, bursa, spleen and thymus) tissues was therefore examined in domestic fowl. In all tissues, a 4.4 kb GHR gene transcript that encodes the full-length GHR was identified. The abundance of this transcript was inversely related to endogenous GH status; it was lower in males with high circulating concentrations of GH and higher in females with lower basal concentrations of plasma GH. The abundance of this transcript was also rapidly downregulated in response to a

bolus systemic injection of recombinant chicken GH, designed to mimic an episodic burst of endogenous GH release. This autoregulatory response was observed within 2 h of GH administration and was of greater magnitude in the brain than in peripheral tissues. Intracerebroventricular injections of GH also downregulated GHR gene expression in the brain, although hepatic GHR transcripts were unaffected 24 h after central administration of GH. In contrast, the induction of hyposomatotropism by passive GH immunoneutralization increased the abundance of the GHR transcript in the thymus, but not in other central (brain) or peripheral (bursa, liver) tissues. GH is not the sole regulator of GHR abundance, however; hypersomatotropism induced by hypothyroidism was associated with an increase in GHR mRNA. The expression of the GHR gene in the domestic fowl would thus appear to be autoregulated by GH in a tissue-specific way.

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Introduction

Growth hormone (GH) regulates numerous cellular functions by binding to growth hormone receptors (GHRs) and activating intracellular signalling cascades (Herington 1994, Kelly *et al.* 1994). A common receptor is believed to mediate these tissue-specific effects, suggesting that post-receptor signalling molecules or tissue sensitivity to GH may differ between tissues. Tissue sensitivity depends upon the abundance of GHRs, thus alterations in GHR gene transcription or translation could contribute to the specificity of GH action. GHR regulation by GH itself may be a particularly important determinant of GH action, as it would enhance or reduce the effect of a particular dose of GH. The influence of chicken GH (cGH) on its receptor, however, remains controversial and unclear (Harvey & Hull 1995).

In broiler fowl, hepatic GH-binding activity has been reported to be unaffected (Vanderpooten *et al.* 1991, Moellers & Cogburn 1995) or downregulated (Vanderpooten *et al.* 1991, Moellers & Cogburn 1995) by

GH administration, which reportedly has no effect on hepatic GHR gene expression (Cogburn *et al.* 1997). The abundance of hepatic GHR transcripts has also been shown to be inversely related to circulating concentrations of GH during ontogeny (Burnside & Cogburn 1992). The relationship between GH and the GHR and the GHR gene may, however, depend on endogenous GH status, as hepatic GH-binding sites are downregulated by GH in hypophysectomized, but not in intact, birds (Vanderpooten *et al.* 1991). Similarly, other studies have demonstrated the downregulation of these sites in intact birds given intermittent injections, rather than continuous infusions of GH (Moellers & Cogburn 1995). The influence of GH on hepatic GHR gene expression was therefore further evaluated in the present study, in birds with low circulating concentrations of GH.

The liver is not the only target site for GH. Indeed, in the chicken, GHRs have recently been demonstrated in the central nervous system (Hull *et al.* 1993) and immune system (Hull *et al.* 1996c), suggesting novel roles for GH in neural and neuroendocrine function (Buntin & Figge

1989, Lea *et al.* 1990) and in cellular and humeral immunity (Marsh 1992, Hull *et al.* 1996a). These non-hepatic GHRs may also be dependent upon GH status, but may be regulated differently than the hepatic GHR. This possibility was first suggested by Frick *et al.* (1990), who showed that GHR mRNA was increased in rat muscle and liver, but decreased in fat, by hypophysectomy. Tissue differences in GHR autoregulation would enable a greater variation in tissue responses to the same GH bolus, thus augmenting the tissue specificity of GH action; however, the regulation of the GHR gene in non-hepatic sites has not been investigated. We have therefore examined the possibility that the abundance of neural and immune GHR transcript may be autoregulated, and compared this with the autoregulation of the hepatic GHR mRNA.

Materials and Methods

Birds and tissues

Adult White Leghorn chickens were used in most studies, as the abundance of hepatic GHR mRNA is upregulated during ontogeny and greatest in old birds with low circulating concentrations of GH (Harvey *et al.* 1991, Burnside & Cogburn 1992); inhibitory effects of GH on GHR mRNA abundance would thus be readily detectable in tissues of adult fowl. The birds were killed by cervical dislocation, and central (whole brain, hypothalamus or extrahypothalamic brain) and peripheral (liver, spleen bursa, or thymus) tissues were rapidly dissected out, frozen in liquid nitrogen and stored at -80°C until required for northern blot analysis.

Influence of exogenous GH

Peripheral administration of GH In poultry, the secretion of GH is pulsatile, with an episode of GH release every 1.5–2 h (Vasilatos-Younken & Zarkower 1987, Bacon *et al.* 1989). Hepatic GH-binding activity is decreased by pulsatile, but not continuous, administration of GH (Moellers & Cogburn 1995). The possibility that a phasic increase in the circulating concentration of GH might autoregulate GHR gene expression in chickens was therefore examined, especially as hepatic GHR abundance and GHR gene expression in the rat are dynamically regulated by pulsatile episodes of pituitary GH secretion (Bick *et al.* 1992, Hull *et al.* 1996b).

Adult males ($n=8$ per group) were injected i.m. with recombinant chicken GH (rcGH; Amgen, Thousand Oaks, California, USA) (100 $\mu\text{g}/\text{kg}$ body weight) or with 0.9% w/v NaCl vehicle (1 ml/kg) and tissues were collected 2 h later. The administration of GH by this route results in a rapid increase in circulating GH, which peaks after 40 min and declines exponentially thereafter (Harvey

1993). For comparative purposes, tissues were similarly collected 2 h after GH administration from birds that had been injected with GH once daily for 14 days.

Intracerebroventricular administration of GH Because the GH gene is expressed in the brain, GHRs in neural tissues may be modulated by GH of pituitary and neural origin. The possibility that alterations in central GH status might differentially affect central or peripheral GHR transcripts was therefore examined by injecting 10 or 100 μg ovine GH (oGH; NIADKD, Bethesda, MD, USA) ($n=6$) or 0.9% w/v saline vehicle ($n=6$) into the third cerebral ventricle of adult male White Leghorn chickens. Membrane binding sites for oGH have been demonstrated in the chicken hypothalamus (Attardo & Harvey 1990) and similar intracerebroventricular (i.c.v.) injections of oGH have previously been shown to alter brain neurochemistry in domestic fowl (Lea & Harvey 1993). The birds were killed after 24 h and the liver and brain extracted and quick-frozen in liquid nitrogen.

Influence of endogenous GH

GH immunoneutralization The continuous infusion of GH into young broilers does not reduce GH binding to hepatic sites (Moellers & Cogburn 1995), possibly because their high endogenous concentrations of GH impair their sensitivity to exogenous GH. The effect of hypsomatotropicism on GHR gene expression was therefore examined in rapidly growing White Leghorn chickens. [In rats, hepatic GHR gene expression is more sensitive to chronic than acute changes in GH status (Carmignac *et al.* 1993, Baumbach & Bingham 1995).] Endogenous GH concentrations were reduced by passive GH immunoneutralization, which has previously been shown to induce growth retardation in rapidly growing birds (Scanes *et al.* 1977). Immature (5-week) chickens were injected i.m. daily for 10 days with a high-titre antiserum (0.5 ml) raised against cGH (Harvey & Scanes 1977) or with normal rabbit serum (NRS; $n=8$ per group) and the birds were killed 2 h after the last injection. The titre of the injected antibody was determined by its ability specifically to bind ^{125}I -labelled cGH (Harvey & Scanes 1977). The antibody had an affinity constant (K_a) of 3.5×10^{-9} M and a binding capacity of 6.0×10^{-8} M.

Hypothyroidism Endogenous concentrations of GH in chickens are markedly affected by thyroid status. Hypothyroidism, for instance, is a well-known stimulus for GH secretion (Harvey *et al.* 1988). The possibility that the induction of GH release in hypothyroid birds might downregulate tissue GH receptors was therefore examined. Adult male chickens were made hypothyroid by daily i.p. injections of thiourea (50 mg/kg), for 3 weeks. The birds were killed 2 h after the final injection and

Figure 1 Northern blot analysis of GHR gene expression in chicken tissues. RNA moieties from liver, hypothalamus (hypothal.), extrahypothalamic brain (brain), bursa, and spleen are shown hybridized to a chicken GHR cDNA probe.

central and peripheral tissues were collected. Serum GH concentrations were determined by RIA (Harvey & Scanes 1977).

Gender Endogenous concentrations of GH in the plasma of adult males are known to be greater than those in laying hens (Harvey *et al.* 1991). The possibility that male and female birds might differ in GHR gene expression was therefore examined and correlated with plasma GH, as determined by RIA (Harvey & Scanes 1977).

Northern blot analysis

Total cellular RNA was extracted from central and peripheral tissues using a commercial method, according to the manufacturer's instructions (RNA Now, Biocan Scientific, Mississauga, Ontario, Canada). In some studies, tissues from paired birds were pooled to facilitate analysis, especially as GHR abundance in non-hepatic tissues was expected to be low (Hull *et al.* 1993).

After extraction, the RNA was subjected to northern blot analysis as described previously (Render *et al.* 1995b), using a complementary DNA fragment (cGHR) (provided by Dr W Baumbach, American Cyanamid, Princeton, NJ, USA) encoding the majority of the coding region of the chicken GHR (cGHR). GHR mRNA content was quantified by laser densitometry and corrected for loading error by comparison with the hybridization of a GAPDH cDNA probe (provided by Dr W Baumbach). Autoradiograph exposure time was optimized for the RNA from each tissue and differences in transcript abundance between the different treatment groups were deter-

mined by one-way ANOVA and the least significant difference test. Transcript abundance was not compared between different tissues, in view of these differences in autoradiographic exposure time.

Results

Tissue GHR mRNA

A major transcript of 4.4 kb, hybridizing to the cGHR cDNA probe, was observed consistently in RNA extracted from liver, hypothalamus, extrahypothalamic brain regions, thymus, bursa and spleen (Fig. 1). Table 1 summarizes the changes in the abundance of this transcript in relation to the various factors tested. Smaller 1.0 kb and 2.8 kb transcripts also hybridized with the GHR cDNA probe (data not shown). The abundance of these transcripts was not quantified, however, as their detection often required considerable overexposure of the autoradiograph.

Influence of exogenous GH

The abundance of the hepatic 4.4 kb transcript was reduced by 25% 2 h after a single peripheral injection of cGH, compared with that in saline-injected chickens ($P<0.001$) (Fig. 2). GHR transcripts in the brain and spleen were also reduced in GH-treated birds ($P<0.001$), the magnitudes of the reductions (40% in spleen, 60% in brain) being significantly greater ($P<0.001$) than those in the liver.

The abundance of the 4.4 kb GHR transcript in hypothalamic and extrahypothalamic tissues was similarly reduced (by 28% ($P<0.001$) and 32% ($P<0.001$) respectively) 2 h after the last GH injection in birds pretreated for 13 days with the same daily dose of cGH (Fig. 3). The mean reduction in whole brain (hypothalamic and extrahypothalamic) GHR mRNA was, however, less ($P<0.001$) than that in transcript abundance induced by a single GH injection. Moreover, whereas hepatic GHR mRNA was reduced after a single GH injection, it was unaffected after GH pretreatment (Fig. 3).

Table 1 Changes in GHR mRNA abundance in relation to various factors tested

	GHR mRNA (% control)					
	Liver	Brain	Hypothal.	Spleen	Thymus	Bursa
Treatment						
GH (2 h)	75	60		40		
GH (14 days)	(NS)	68	72			
GH (i.c.v.)	(NS)	75				
GH antiserum	(NS)	(NS)			141	(NS)
Hypothyroid	147	121		111		
Females (% males)	111	180		133		

Hypothal., Hypothalamus; (NS), changes were not significant – all other values were significantly different ($P<0.05$) from their respective controls.

Figure 2 Abundance of the 4.4 kb GHR transcript in the liver, spleen and brain of chickens 2 h after an injection of 0.9% saline (Control, ■) or rcGH (GH, □). GHR mRNA abundance was quantified by laser densitometry and expressed relative to the abundance of GAPDH mRNA. To facilitate comparisons between tissues, data are presented as a percentage of control values (mean $\pm$ S.E.M.). *** $P < 0.001$ compared with Control (ANOVA).

Figure 3 (a) The abundance of the 4.4 kb GHR transcript in the hypothalamus, extrahypothalamic brain (brain) and liver after 14 daily i.m. injections of cGH (GH, □) or 0.9% saline (Control, ■). Birds were killed 2 h after the final injection. GHR mRNA abundance was quantified by laser densitometry and expressed relative to the abundance of GAPDH mRNA in arbitrary units. To facilitate comparisons between tissues, data are presented as a percentage of control values (mean $\pm$ S.E.M.). * $P < 0.05$, ** $P < 0.01$ compared with Control (ANOVA). (b) Northern blots of liver (four selected lanes for each treatment group) and hypothalamus (three lanes for each treatment group, each consisting of two or three pooled hypothalami), for illustrative purposes.

The abundance of the brain 4.4 kb GHR transcript was also reduced ($P < 0.05$) 24 h after the i.c.v. injection of 10 or 100 $\mu\text{g/kg}$ GH, but the abundance of the hepatic GHR transcript was unaffected in both groups (Fig. 4).

Figure 4 Abundance of the 4.4 kb GHR transcript in the liver, brain, bursa and thymus after five daily injections of a high-titre GH antiserum (GH antibody, □) or of normal rabbit serum (Control, ■). GHR mRNA abundance was quantified by laser densitometry and expressed relative to the abundance of GAPDH mRNA in arbitrary units. To facilitate comparisons between tissues, data are presented as a percentage of control values (mean $\pm$ S.E.M.). *** $P < 0.001$ compared with antibody treated group (ANOVA).

Influence of endogenous GH

Daily administration of a high-titre GH antiserum (αGH) for 10 days reduced growth rate by 18% (body weight gain 175.5 ± 9.4 g in NRS controls and 148 ± 7.8 g in αGH -treated birds; $P < 0.05$). The ability of the antibody to immunoneutralize endogenous GH was demonstrated by the persistence of GH-binding activity in the serum from all the antibody-injected birds (whereas serum from NRS-treated birds did not bind labelled cGH, 50% of the ^{125}I -cGH tracer was bound with serum from the treated birds at final dilutions of 1:128–1:1024). However, while GH immunoneutralization resulted in a significant increase in GHR mRNA abundance in the thymus ($P < 0.001$), it had no effect on GHR transcript abundance in the brain, liver or bursa (Fig. 5).

As expected, daily injections of PTU for 14 days induced hypothyroidism, as indicated by thyroid enlargement in thiourea-treated birds (thyroid weight 0.400 ± 0.023 g) compared with saline-treated birds (0.240 ± 0.011 g; $P < 0.001$) and chronically increased ($P < 0.01$) circulating concentrations of GH (12.6 ± 2.4 $\mu\text{g/l}$ in controls; 20.6 ± 2.4 $\mu\text{g/l}$ in PTU-treated birds). The abundance of the 4.4 kb transcript was increased ($P < 0.05$) in hypothyroid birds in both central (brain) and peripheral (liver and spleen) tissues (Fig. 6).

Basal circulating concentrations of GH in adult male birds were increased compared with those in adult females (plasma GH 13.7 ± 1.7 $\mu\text{g/l}$ in males and 7.8 ± 1.3 $\mu\text{g/l}$ in females). The abundance of the major GHR transcript in male tissues was consistently ($P < 0.05$ or less) lower than in tissues from females (Fig. 7). The gender difference was particularly marked in the brain and spleen GHR mRNA,

Figure 5 Abundance of the 4.4 kb GHR transcript in the brain and liver 24 h after a single injection into the third cerebral ventricle of cGH (i.c.v. GH, □) (10 or 100 µg) or 0.9% saline (Control, ■). Both doses of injected GH were equally effective; for clarity of presentation, the data from both groups are combined. GHR mRNA abundance was quantified by laser densitometry and expressed relative to the abundance of GAPDH mRNA in arbitrary units. To facilitate comparisons between tissues, data are presented as a percentage of control values (mean ± S.E.M.). * $P < 0.05$ compared with Control (ANOVA).

Figure 6 Abundance of the 4.4 kb GHR transcript in the liver, brain and spleen of euthyroid (■) or hypothyroid (□) birds receiving daily injections of 0.9% (w/v) saline or thiourea (respectively) for 21 days. GHR mRNA abundance was quantified by laser densitometry and expressed relative to the abundance of GAPDH mRNA in arbitrary units. To facilitate comparisons between tissues, data are presented as a percentage of control values (mean ± S.E.M.). * $P < 0.05$, ** $P < 0.01$ compared with Hypothyroid (ANOVA).

in which GHR transcript abundance in females was 180% and 133%, respectively, of that in males ($P < 0.001$). In contrast, the abundance of the GHR mRNA in the livers of males was only 11% less than that in the livers of females, and the magnitude of the difference was less ($P < 0.05$) than the gender-related reduction in transcript abundance in the brain and spleen.

Figure 7 Relative abundance of the 4.4 kb and 2.8 kb GHR transcripts in adult male (■) and female (□) chickens. GHR mRNA abundance was quantified by laser densitometry and expressed relative to the abundance of GAPDH mRNA in arbitrary units. To facilitate comparisons between tissues, data are presented as a percentage of control values (mean ± S.E.M.). * $P < 0.05$, *** $P < 0.001$ compared with females (ANOVA).

Discussion

These studies demonstrate, for the first time, that GH downregulates GHR gene transcripts in the brain, immune system and, to a lesser extent, the liver, of adult chickens. These results contrast with the findings of similar studies in rats, in which GHR mRNA abundance in these tissues was increased in response to a single exogenous GH bolus (Hull *et al.* 1996b). This tissue- and species-specificity of GHR mRNA autoregulation may enable GH to exert disparate, species- and tissue-specific cellular responses via a single class of GHR.

The abundance of hepatic GHR transcripts was decreased within 2 h after a single GH injection. In contrast, Cogburn *et al.* (1997) recently concluded that GHR gene expression in immature broiler fowl was insensitive to exogenous infusions or injections of GH. It is possible, however, that the lack of GHR autoregulation in that study was due to the high endogenous plasma concentration of GH and relatively low abundance of hepatic GHR mRNA in rapidly growing birds (Harvey *et al.* 1991, Burnside & Cogburn 1992). Indeed, as endogenous GH secretion in adults is less than that in young birds, tissue GHR transcripts may be upregulated and therefore more sensitive to GH autoregulation. This possibility is supported by the ability of exogenous GH to downregulate GH-binding sites in hypophysectomized chicks, but not in intact chicks (Vanderpooten *et al.* 1991). Adult birds may therefore provide a better model than young birds for the assessment of GHR autoregulation.

The magnitude of GH-induced GHR mRNA downregulation was, interestingly, greater in tissues that synthesize GH (brain and immune tissues) than in the liver, which does not produce GH (Render *et al.* 1995a, b). For

instance, chronic peripheral administration of GH resulted in a 38% reduction in hypothalamic GHR mRNA, but only an 8% reduction in hepatic GHR mRNA. This enhanced sensitivity of central and immune GHRs to autoregulatory influences would suggest that GH has an important role in these tissues, as high concentrations of GH would reduce GHR abundance and thus protect the tissue from excess GH stimulation. Inhibitory autoregulation of central GHRs may, for instance, protect the hypothalamus from excess GH-induced negative feedback (Lea *et al.* 1990), as somatostatinergic tone is increased (Harvey *et al.* 1990) and catecholamine synthesis is decreased (Harvey 1993, Lea & Harvey 1993) in response to peripheral GH. Although factors regulating immune GH secretion have not been elucidated, similar mechanisms could occur in these tissues.

GH-induced GHR mRNA downregulation in central and immune tissues may also prevent excess activation of other GH-dependent processes. Immune GHRs may, for instance, mediate stimulatory GH effects on the growth and development of lymphoid organs (Marsh 1992) and cells (Haddad & Mashaly 1991, Johnson *et al.* 1993). GH-induced GHR downregulation in the avian spleen and thymus may thus protect the animal from inappropriate or excess activation of the immune system.

Although GH could easily gain access to immune tissues, the mechanism by which peripheral GH alters brain receptors has not been elucidated in any species (Harvey *et al.* 1993). It is, however, becoming increasingly apparent that peripheral GH can gain access to the brain and alter the functioning of many brain regions, including the cerebral hemispheres (Lopez-Fernandez *et al.* 1996). GH may circumvent the blood-brain barrier via the choroid plexus or other circumventricular organs, as GHRs are present in the mammalian choroid plexus (Lai *et al.* 1991, Garcia-Aragon *et al.* 1992) and some, but not all, studies revealed uptake of radiolabelled GH from the periphery into the brain (Stern *et al.* 1976, Pacold *et al.* 1978, Hojvat 1982). Alternatively, GH may enter the brain from the pituitary gland, via retrograde transport up the hypophyseal stalk (Sato *et al.* 1989) or passage through the short pituitary portal vessels (Oliver *et al.* 1977). It is also possible that peripheral GH does not enter the brain, but acts via intermediary factors such as insulin-like growth factors (IGFs). This mechanism is not likely to have a major role, as IGF-I does not affect hepatic GHR expression in rats (Carmignac *et al.* 1993).

The possibility that GH may directly affect central GHRs is further supported by the ability of centrally injected GH to reduce brain GHR expression. Curiously, peripheral and central injections of GH stimulate and inhibit (respectively) central GHR expression in rats (Bennett *et al.* 1995). Bennett *et al.* (1995) suggested that this differential regulation of central GHRs by central and peripheral GH may represent the dose-dependent nature of GHR autoregulation; the greater concentration of

i.c.v.-injected GH could be inhibitory, whereas the lower i.c.v. concentration resulting from peripheral injection could be stimulatory. In contrast, both central and peripheral injections of GH reduced brain concentrations of GHR mRNA in chickens. This may indicate a lesser degree of dose-dependency in chickens, or that peripheral GH may be affecting central GHRs by an indirect mechanism in mammals, but a direct mechanism in chickens.

Peripheral and central GHR expression would appear to be constitutively inhibited by normal concentrations of endogenous GH, as GHR mRNA was more abundant in female chickens, which are hypsomatotropic compared with male chickens. However, endocrine factors other than GH also have a determining role in GH transcript abundance. A decrease in circulating thyroid hormone status, for instance, increases GHR mRNA abundance, despite a parallel increase in circulating GH. GHR transcript regulation is thus complex and multifactorial.

In summary, GH causes a rapid and long-lasting reduction in GHR expression in central and peripheral tissues of the domestic fowl. This inhibitory interaction may result in some degree of GH resistance; thus strategies to increase receptor expression may be necessary to improve GH responsiveness in this species.

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