Age-related changes in prolactin and growth hormone release from pituitary glands in vitro

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Abstract. The basal release of prolactin from cockerel anterior pituitary glands in vitro declined between 1 and 7 weeks of age, to a level less than that released by pituitary glands from 18 week old (adult) cockerels and hens. Basal growth hormone (GH) release increased between 1 and 7 weeks of age but had declined in adults to a level similar to that released from 4 weeks old cockerels. The responsiveness of the pituitary gland to hypothalamic stimulation, using hypothalami from 8 week old broiler fowl, was also age-related. Prolactin release was considerably higher from pituitaries of 1 week old cockerels compared to the other age groups. Stimulation of GH release by the hypothalamus was higher from pituitaries of both 1 and 7 week old cockerels compared to the other groups of birds. The increase in release of prolactin following incubation with thyrotrophin releasing hormone (TRH) declined between 1 and 7 weeks, but increased slightly in adult birds, whereas the increase in release of GH following TRH was higher from pituitaries of both 1 and 7 week old cockerels. Hypothalamic prolactin (Prl) releasing activity, measured as the ability of the hypothalamus to stimulate hormone release from 8 week old broiler fowl anterior pituitary glands, declined with the age of the donor cockerels. The hypothalami from adult hens secreted significantly more Prl releasing activity than did adult cockerel hypothalami. The secretion of GH releasing activity decreased markedly with the age of the donor bird.

These results suggest that maturational patterns of hormone secretion in fowl are partly due to changes in autonomous hormone release, to changing patterns of hypothalamic activity and to differences in pituitary responsiveness to provocative stimuli.

In birds age-related changes in prolactin (Prl) and growth hormone (GH) secretion have been demonstrated in vivo. The circulating GH level is high in young chicks, declines during maturation and is very low in adult birds (e.g. Burke & Marks 1982; Harvey 1983). Plasma Prl levels also tend to be highest in young birds, but are lowest prior to sexual maturation, the onset of which is accompanied by an elevation in the Prl concentration (Harvey et al. 1979a; Burke & Marks 1982). The mechanisms responsible for these developmental patterns are unknown, although may involve age-related changes in the metabolism (Scanes & Lauterio 1984) or secretion of the pituitary hormones.

The release of both Prl and GH from the avian pituitary occurs in response to stimulatory hypothalamic hypophysiotropic factors (a growth hormone releasing factor (GRF) and a prolactin-releasing factor (PRF)) and is impaired by inhibitor factors (a growth hormone release inhibiting hormone, somatostatin or SRIF, and a prolactin-inhibiting factor, possibly dopamine) (Chadwick & Hall 1983; Harvey 1983). The age-related patterns in plasma Prl and GH concentrations may therefore reflect maturational differences in the balance of these stimulatory and inhibitory hypothalamic factors or developmental changes in pituitary sensitivity to hypothalamic stimulation or inhibition. The demonstration of a maturational increase in the sensitivity of rat somatotrophs to SRIF inhibition provides evidence for such a mechanism in mammalian species (e.g. Rieutort 1981; Khorram et al. 1983), while the finding of diminished in vivo GH responses of adult fowl to provocative stimuli (e.g. Harvey et al. 1981), suggests a similar mechanism in the fowl. The possibility that alterations in hypo-
thalamic releasing activity or in pituitary respon-
siveness to hypothalamic stimulation may be re-
ponsible for the previously observed changes in
the circulating GH and Prl concentrations in grow-
ing chicks has therefore been examined, in vitro, in
the present study.

Materials and Methods

One day old chicks (Thorber 909 strain) were maintained
under an 18 h light:6 h dark photoperiod with free
access to food and water. The birds were kept at 30°C
when one day old, although the environmental tem-
perature was gradually reduced to 20°C by the time chicks
were 4 weeks old, in accordance with commercial prac-
tice. Groups of birds which had reached 1, 4, 7 and
18 weeks of age were then killed on the same day, by
decapitation, together with a group of adult (18 week
old) hens. The anterior pituitary glands and mediobasal
hypothalami were then dissected from the heads and
collected into ice-cold Medium 199 (Wellcome Labora-
tories). Each pituitary gland was bisected longitudinally,
the hemipituitary glands weighed and pre-incubated for
60 min in Medium 199, at 39°C under an atmosphere of
95% O2:5% CO2. The media were then aspirated and
replaced by fresh media containing the experimental
media and the tissues were incubated for a further
4 h to permit hormone release to reach an equilibrium
(Hall & Chadwick 1983). Histological examination of
pituitary glands incubated under such conditions has
demonstrated that the incubation media completely
penetrates the largest (adult) glands as revealed by the
presence of viable actively secreting somatotrophs and
lactotrophs within the central region (Tai 1976; El
Tounsy 1979). During incubation the hemipituitaries
were incubated solely in media or together with single
whole hypothalami collected from the heads of 8 week
old slaughter house chickens (Hall et al. 1984c) or
in medium containing thyrotrophin releasing hormone
(TRH, Reckitt & Colman) at a concentration (10⁻⁷ M)
that maximally stimulates in vitro Prl and GH release
(Hall et al. 1984c). Bacitracin (10⁻⁴ M) was also added to
the incubation media to prevent tissue degradation of
hypothalamic releasing factors (McKelry et al. 1970).
Since TRH and hypothalamic co-incubations stimulate in
vitro Prl and GH release (Hall & Chadwick 1983; Harvey
1983) both basal and stimulated hormonal release from
the pituitary glands of old and young chickens were
therefore determined in this study. To determine age-
related changes in the prolactin (PRA) or growth hor-
mones (GRA)-releasing activity of the chicken hypothala-
umus (rather than releasing factor content), the medio-
basal hypothalami were incubated for 4 h with hemipitui-
tary glands obtained from the heads of the freshly killed
8 week old broiler fowl (Hall et al. 1984c), the contra-
lateral hemipituitary glands being incubated solely in
Medium 199. At the end of the incubation period the
media samples were separated, appropriately diluted and
stored at −20°C to await analysis.

Concentrations of Prl and GH in the media samples
were determined by specific, homologous radioimmuno-
assays (Harvey & Scares 1977; Lea et al. 1982). All the
samples were assayed together, to avoid inter-assay vari-
ation. Statistical differences in the results were determined
by Student’s t-test or by analysis of variance and Duncan’s
Multiple range test, wherever appropriate.

Results

Data on the basal and stimulated release of Prl and
GH from the pituitary glands of young and old
birds are summarised in Fig. 1.

The basal release of Prl from unstimulated pitui-
tary glands fell (P < 0.05) between 1 and 7 weeks of
age. Adult cockerels and adult hens had similar
basal levels of Prl release, the mean concentration
of which was higher (P < 0.05) than that released from
the pituitaries of 7 week old fowl. At all ages
co-incubation with broiler hypothalami or incuba-
tion in 10⁻⁷ M TRH resulted in increased (P < 0.01)
Prl release. The stimulation of Prl release by hypo-
thalamic co-incubation was consistently greater
than that induced by TRH, significantly so (P <
0.05) with respect to the pituitaries from 1, 4 and
7 week old fowl. Although the pituitaries from 4,
7 and 18 week old birds did not differ significantly
in the absolute or relative amounts of Prl released
in response to TRH or hypothalamic stimulation
the pituitaries from 1 week old chicks were more
(P < 0.01) responsive, especially to hypothalamic
stimulation. Whereas basal and hypothalamic in-
duced Prl release were of similar magnitude for the
pituitaries from 4, 7 and 18 week old birds, hypo-
thalamic co-incubation with pituitaries from 1 week
old chicks resulted in an 180% increase in Prl
release, compared with the basal level. The Prl
response of the neonatal pituitaries to TRH stimu-
lation was also greater (P < 0.05) than that elicited
from the pituitaries of 4 and 7 week old fowl.

The basal release of GH increased (P < 0.01)
between 1 and 7 weeks of age, but subsequently
declined (P < 0.01) to levels of growth hormone
release in the adults, which were comparable with
those in 1 and 4 week old birds. The co-incubation
with broiler fowl hypothalami significantly (P <
Age-related changes in the in vitro release of Prl and GH from chicken pituitary glands. On the left-hand side the shaded bars represent basal hormone release, the striped bars represent the increase in hormone release (in comparison with pituitaries incubated in medium alone) from pituitaries co-incubated with a single mediobasal hypothalamus and the solid bars represent the increase in the hormone release following incubation in 10^{-7} M TRH. On the right-hand side the increase in hormone release following hypothalamic co-incubation (hatched bars) or TRH stimulation (open bars) is expressed relative to the mean basal level of Prl or GH release. Means ± SEM (n varied between 5 and 8).

0.05) increased the level of GH release from the pituitaries of 1, 4 and 7 week old chicks, but not from the pituitary glands of adult birds, in which the stimulatory response was more variable. At all ages, however, the stimulation of GH release in response to hypothalamic stimulation was less than that induced by TRH, significantly ($P < 0.05$) so for 1, 4 and 7 week old birds. Incubation with TRH consistently increased ($P < 0.05$) the level of GH release and by a similar magnitude for 4, 7 and 18 week old birds. The GH response in 1 week old birds to TRH stimulation was greater ($P < 0.01$) than that evoked at other ages and was unusual in that it was of greater magnitude (by 250%) than the basal level of GH release.

Data on the GRA and PRA of the chicken hypothalami are shown in Fig. 2.

The basal release of GH from the 8 week old broiler fowl pituitaries (0.80 ± 0.05 (n = 37) µg/mg) was consistently less ($P < 0.001$) than that released from the pituitaries of the Thornber chickens, as was the basal release of Prl (21.7 ± 0.72 (n = 39) ng/mg). Although the basal levels of Prl and GH release were similar for each group of broiler fowl pituitaries (data not shown), significant differences were observed in the magnitude of the GH and Prl responses upon incubation with hypothalami derived from 1, 4, 7 and 18 week old birds. Whereas co-incubation with hypothalami from 7 and 18 week old fowl had no effect on GH release, in-
It is now well established that the circulating GH level is high in young birds, reaching a peak between 2 and 8 weeks of age, and declines with advancing maturity (Harvey et al. 1979a; Burke & Marks 1982). Age-related differences in the plasma GH level are not, however, reflected by corresponding alterations in autonomous basal GH release (Fig. 1), although the slight increase between 1 and 7 weeks of age coincides with the observation of maximal plasma GH concentrations. This is somewhat surprising, in view of the reduced number of somatotrophs in the adult pituitary gland (Tai 1976; Malamed et al. 1984) and may indicate that the adult somatotrophs contain comparatively larger stores of releasable GH or that basal GH synthesis and release occur faster than in younger birds.

The low basal level of plasma growth hormone in adult birds partly results from an accelerated clearance rate (14 min in 18 week old birds, 21 min in 3 week old chicks, unpublished data and Scanes & Lauterio 1984), but the findings of the present study demonstrate that this also results from an age-related decline in pituitary responsiveness to provocative stimuli and to a decline in the GRA of the hypothalamus. Scanes & Gross (1983) similarly observed that dispersed pituitary cells from 2 week old birds are more responsive to TRH stimulation, in terms of GH release, than the cells from adult pituitary glands.

The diminished responsiveness of adult birds to TRH and hypothalamic stimulation appears to be in agreement with in vivo studies (e.g. Harvey et al. 1981; Harvey 1983), in which the attenuated GH responses of adult birds to exogenous TRH were thought to be due to endogenous somatostatin inhibition. However, while a decrease in somatotroph sensitivity to provocative stimuli occurs during the maturation of the fowl, this is unlikely to be responsible for the diminished in vivo GH responses of adult birds to TRH (Harvey et al. 1981), which were compared with the GH responses in 4 week old chicks. At this age pituitary sensitivity to both TRH and hypothalamic stimulation is similar to that in adults. The decline in the sensitivity of the somatotrophs to provocative stimulation therefore paradoxically occurs at a time when maximal plasma GH concentrations are observed. The rapid decline in the sensitivity of the pituitary somatotrophs to provocative stimulation may occur as a result of a reduction in the number or binding affinity of the receptors involved or

Increased \((P < 0.01)\) GH release was induced by co-incubation with hypothalami from 1 and 4 week old birds. Similarly, although co-incubation with hypothalami consistently increased \((P < 0.01)\) Prl release the response with hypothalami from 1 and 4 week old cockerels was greater \((P < 0.05)\) than that evoked by the hypothalami from 7 and 18 week old cockerels. The PRA of the adult cockerels was also less \((P < 0.05)\) than the PRA of the hypothalami derived from adult hens.

**Discussion**

These results clearly demonstrate age-related changes in the pituitary and hypothalamic function of domestic fowl.

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**Fig. 2.**

Age-related changes in hypothalamic releasing activity. Data are expressed relative to the level of Prl (●) or GH (○) released from control pituitaries incubated solely in incubation medium. Single, whole mediobasal hypothalami from 1, 4, 7 and 18 week old chickens were incubated with pituitary glands from 8 week old broiler fowl. Means ± SEM \((n = 8)\).
from functionally impaired stimulus-coupling (Tixier-Vidal & Gourjdj 1981) or from a reduction in the size of the releasable pool of pituitary GH.

In contrast with pituitary sensitivity, GRA of the chicken hypothalamus declined at a time (between 7 and 18 weeks of age) when the circulation GH level declines. However, rather than being indicative of a loss of hypothalamic GRA in the adults, these results probably indicate, as in rats (Rietort 1981; Khorram et al. 1983), the development of inhibitory hypothalamic control (Harvey 1983) in the neuroendocrine regulation of GH secretion. It is also possible that maturational changes in hypothalamic neurotransmission, as occur in mammalian species (Makman et al. 1979; Meites 1982) may contribute to the loss of GRA in adult hypothalamus; the addition of acetylcholine, serotonin, dopamine and gamma aminobutyric acid (GABA) to incubation media blocking in vitro GH responses to releasing stimuli (Hall et al. 1984a,b,c). Several of these neurotransmitters have been detected in the avian hypothalamus (Chadwick & Hall 1983) and their presence may counteract the stimulatory effect of hypothalamic releasing factors on in vitro GH release. Since dopamine and GABA also blunt prolactin responses to provocative stimuli (Hall et al. 1984b,c) a maturational increase in the dopamine or GABA content of the cockerel hypothalamus may also account for the observed age-related decline in the PRA of the hypothalamus.

In the present study the basal and stimulated release of prolactin tended to decline between 1 and 7 weeks of age. This decline is similar to the post-natal decline in the basal plasma Prl concentration (Harvey et al. 1979b) and the reduction in the in vivo Prl response to hypothalamic stimulation observed between 3 and 8 weeks of age (Harvey et al. 1979b). The increase in basal Prl release between 7 and 18 weeks of age may reflect the onset of gonadal development and the sensitising effect of oestradiol and testosterone on basal Prl release (Hall et al. 1984d,e) and the increased PRA in the hypothalamus of the adult hens may similarly result from oestradiol sensitisation (Hall et al. 1984d).

Since the magnitude of the in vitro Prl and GH response to hypothalamic stimulation does appear to depend upon the age and physiological condition of the donor birds, maturational differences in pituitary and hypothalamic function may contribute to the variable effects of hypothalamic stimulation on the in vitro release of Prl and GH from avian pituitary glands (e.g. Hall et al. 1984c; Proudman & Opel 1983; Fehr et al. 1982). In studying the hypothalamic control of Prl and GH secretion, the age of the donor animals should therefore be closely defined.

References
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