drug is administered just before the 'critical period' in adult rat[10]. However, it has opposite effects when 5-HT synthesis is inhibited 20 h earliers[11], this seems to suggest that although 5-HT synaptic terminals located in the median eminence are involved in the preovulatory processing of information necessary for preparing the critical period.

More recently it was indicated that 5-HT administration does not inhibit ovulation at a central site but acts as peripheral vasoconstrictor preventing the passage of hormones to their target organs[12].

There is some supporting evidence for the results obtained here; treatment of ovariectomized rats with steroids increases hypothalamic tryptophan levels[13] and in the midbrain[14]. Significant ($p < 0.01$) decline in 5-HT level was noticed at 48 h after PMS injection which is presumably equivalent to the critical period for the release of LH. The results obtained here is also supported by the in vitro work which indicate that 5-HT can inhibit gonadotropin release from pituitaries but only in the presence of hypothalamic fragments. More over 5-HT has been shown to stimulate the release of prolactin[4].

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In vitro stimulation of chicken pituitary growth hormone and prolactin secretion by chicken hypothalamic extract

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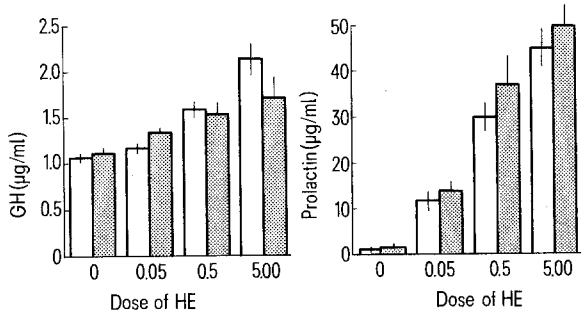
Summary. The effect of an acid extract of chicken hypothalami on the in vitro secretion of prolactin and growth hormone (GH) by dispersed chicken pituitary cells has been investigated. Both prolactin and GH release were stimulated in a dose related manner in the presence of the hypothalamic extract (HE). Somatostatin had no effect on the basal or HE stimulated release of prolactin although it did inhibit the HE induced release of GH.

The existence of growth hormone (GH) releasing activity in the avian hypothalamus has long been established[1-4]. However, this activity has so far only been demonstrated using bioassay[1,2] and electrophoretic[3,4] techniques to measure hypothalamic extract (HE) induced pituitary GH release. Therefore, in view of the fact that chicken HE has recently been found to have no stimulatory effect on the levels of immunoreactive plasma GH in male chickens[5,6], the effect of this extract on the in vitro release of immunoreactive pituitary GH has been investigated in the present study. In addition the effect of this extract on the in vitro secretion of immunoreactive prolactin has also been determined to confirm and extend other studies[7-11] which have established the prolactin releasing activity of avian HE. The influence of incubating somatostatin (growth hormone release inhibitory hormone, GHRIH) alone and together with chicken HE on prolactin and GH release has also been assessed; GHRIH having been found to inhibit in vitro GH release in avian[12] and mammalian[13,14] species and to inhibit prolactin release from incubated rat pituitary cells[15].

Materials and methods. Heads from 8-10-week-old broiler fowl were obtained from a local packing station and the adenohypophyses were removed and collected into ice cold medium 199 (Wellcome Laboratories Ltd) in which they were rapidly transported to the laboratory. Pituitary cells were prepared following the method of Bicknell and Follett[16]. The incubations were carried out in 6.3×0.8 cm disposable polystyrene tubes in a shaking water bath at 39 °C under an atmosphere of 95% O_2, 5% CO_2. To each tube a 0.5 pituitary equivalent was added in 0.5 ml of medium 199 and the test substance then added in a further 0.5 ml of media. After a 2-h incubation the cells were separated from the media by centrifugation (20 min at $1000 \times g$) and the supernatants stored deep frozen prior to assay.

An acid extract was prepared from broiler fowl hypothalami following the method of Follett[17] and was neutralized prior to use and diluted with medium 199. Linear GHRIH was obtained commercially (Digby Chemical Service). Control incubations were in medium 199 alone. Extracts of



Effect of chicken HE, alone (open columns) and in the presence of GHRIH (50 ng/ml; filled columns) on the in vitro release of prolactin and GH from dispersed chicken pituitary cells. Means±SEM (n=5).

other brain tissues have no significant effect on prolactin or GH secretion[9,18]. GH and prolactin concentrations were determined using specific homologous radioimmunoassays[19,20] and statistical differences in the results were determined by Student t-test.

Results and discussion. In a preliminary study the effects of 3 different doses of HE on the release of prolactin and GH was assessed. Treatment with 0.01, 0.02 and 0.2 HE equiv./ml significantly increased prolactin release (86%, 519% and 919% respectively; $p < 0.05$ in each case), as shown in the table. This response was dose-related ($r = 0.774$ [$n = 15$], $p < 0.001$). GH secretion was also significantly enhanced with a dose of 0.2 HE equiv./ml (75%, $p < 0.01$) in a dose related way ($r = 0.646$ [$n = 15$], $p < 0.01$). In a subsequent experiment (figure) GH secretion was again significantly enhanced by 0.5 (44%, $p < 0.05$) and 5.0 (93% $p < 0.01$) HE equiv./ml in a dose related manner ($r = 0.649$ [$n = 15$], $p < 0.01$). In this experiment although the basal release of prolactin was higher ($p < 0.01$) than in the earlier experiment there was again a striking dose related increase in prolactin release ($r = 0.776$ [$n = 15$], $p < 0.001$) with each dose of HE significantly ($p < 0.001$) enhancing the prolactin concentration in the media. There was a 39-fold increase in prolactin release from the pituitary cells incubated with 5 HE equiv./ml.

In this 2nd experiment the effect of GHRIH (50 ng/ml) on the basal and HE stimulated release of GH and prolactin was also investigated. At the dose used it can be seen from the figure that GHRIH had no significant effect on basal, autonomous prolactin release and that in the presence of GHRIH the dose related HE induced rise in prolactin secretion was still evident ($r = 0.683$ [$n = 15$], $p < 0.01$). Somatostatin did not inhibit basal GH secretion nor did it attenuate GH release induced by the lower doses of HE. However, the same dose of GHRIH suppressed the GH release induced by the highest dose of the extract and as a result the dose related GH response to HE stimulation was not seen in the presence of GHRIH ($r = 0.270$ [$n = 15$], $p < 0.1$). This peculiar inhibition pattern suggests that 'basal' GH release may represent non-suppressible leakage from the cells and/or that GHRIH is only effective when immunoreactive GH secretion is markedly enhanced.

The results of this investigation provide further evidence of potent prolactin releasing activity in the chicken hypothalamus and these findings are in agreement with other avian studies in which chicken or pigeon HE induced prolactin release from incubated hemipituitaries has been reported[8-10]. These results are, therefore, in marked contrast with those derived from mammalian studies in which the hypothalamic control of prolactin secretion is predominately inhibitory[21]. In the present study the release of prolactin from incubated pituitary cells was clearly dose-related and was not suppressed by GHRIH. Similarly GHRIH has also recently been reported to have no in vitro effect on the TRH induced release of prolactin from chicken pituitaries[12] and thus suggest that it has little, if any, physiological role in controlling prolactin secretion in birds.

Effect of chicken hypothalamic extract (HE) on the in vitro release of prolactin and GH from dispersed chicken pituitary cells

Dose (HE equiv./ml)	GH (μg/ml ± SEM (n = 5))	Prolactin (μg/ ± SEM (n = 5))
0	1.59 ± 0.15	0.36 ± 0.02
0.01	1.80 ± 0.63	0.67 ± 0.12*
0.02	1.99 ± 0.18	2.23 ± 0.65*
0.20	2.78 ± 0.35*	3.67 ± 0.22**

Significantly different from the controls, * $p < 0.05$, ** $p < 0.001$ (Student's t-test).

The results of the present study also demonstrate that there are factors in the avian hypothalamus which stimulate the in vitro release of immunoreactive GH from the chicken pituitary. These findings are similar to previous in vitro studies in which GH release has been measured by an electrophoretic technique[3,4] but differ from in vivo studies[5] in which the same preparation of chicken HE was found to have no stimulating effect on the circulating levels of immunoreactive GH. These results may, therefore, suggest that the GH releasing activity of the extract may be inactivated in the systemic circulation or that its in vitro stimulatory effect may be blocked in vivo by other hypothalamic substances which may inhibit GH release in vivo but not in vitro. If the latter hypothesis is correct the failure of HE to stimulate GH secretion in vivo may be due to the presence of certain catecholamines (adrenaline and noradrenaline) in the hypothalamus[22]. These catecholamines are known to markedly suppress GH secretion in vivo[23,24] although they have no effect in vitro[5]. Alternatively this lack of in vivo GH response may arise if hypothalamic GHRIH has greater potency in vivo than it does in vitro.

In the present study, synthetic GHRIH had no effect on the basal release of pituitary GH, unlike other avian[3,12] and mammalian[13,14] investigations, although it did suppress the GH response induced by the highest dose of HE (figure). These findings are similar to the in vivo effects of GHRIH on basal and TRH induced release of immunoreactive GH in the chicken[25] and indicate that GH secretion in the fowl may be under dual hypothalamic control.

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