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# Effects on the Secretion of Pituitary Growth Hormone, Thyroid Stimulating Hormone, Luteinizing Hormone and Follicle Stimulating Hormone in Rats Rendered Hyperprolactinaemic by Chronic Treatment with Oestrogen

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*Abstract*

The induction of hyperprolactinaemia in the male rat following chronic high-dose oestrogen administration over 3 months was associated with a significant inhibition of the secretion of growth hormone (GH) ($P < 0 \cdot 02$) thyroid stimulating hormone (TSH) ($P < 0 \cdot 0025$), luteinizing hormone (LH) and follicle stimulating hormone (FSH) (both $P < 0 \cdot 01$). Acute, but not chronic, administration of bromocriptine (1 mg/kg) to these hyperprolactinaemic animals had the effect of normalizing the serum levels of GH and TSH but not those of LH or FSH. While the effects observed on GH, TSH, LH and FSH following induction of hyperprolactinaemia are likely to be consequential to brain actions of prolactin, the present data do not exclude the possibility of direct actions of oestrogen itself.

## Introduction

The induction of hyperprolactinaemia in the male rat following chronic oestrogen administration over 3 months has been shown to result in a highly significant reduction in hypothalamic dopamine (DA) concentration as well as lesser, but significant, reductions in hypothalamic noradrenaline (NA) and serotonin (5-HT) (Smythe and Brandstater 1980). While the hypersecretion of prolactin is a significant endocrine result of oestrogen treatment it might be anticipated that the changes in the status of brain biogenic amines in this situation would also alter the secretion of other pituitary hormones which are thought to be under some degree of central control by these amines. In an early study prior to the availability of radioimmunoassays for pituitary hormones Meyer and Clifton (1956) produced biological data suggesting that the chronic administration of diethylstilboestrol, as well as inducing prolactin-secreting tumours, resulted in reduced pituitary concentrations of growth hormone (GH), thyroid stimulating hormone (TSH), luteinizing hormone (LH) and follicle stimulating hormone (FSH).

The aims of the present study were to investigate the effects of hyperprolactinaemia induced by chronic oestrogen administration on serum concentrations of other pituitary hormones GH, TSH, LH and FSH, and to relate these effects to the changes in hypothalamic DA, NA and 5-HT. Also the effects of the ergot alkaloid bromocriptine on secretion of these other pituitary hormones were examined in the present investigation.

## Materials and Methods

### *Drugs*

The drugs employed in this investigation were: oestradiol valerate (Primogyn Depot, Schering

Pty Ltd, Sydney) and bromocriptine (2-bromo-α-ergocryptine methane sulfonate, CB-154, Sandoz Australia Pty Ltd).

*Experimental Procedures*

*Radioimmunoassays* (RIA)

All rat pituitary hormones were assayed by RIA using reagents (all reference preparations were NIAMDD-RP-1) supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, Rat Pituitary Hormone Program, Bethesda, Md. The upper and lower limits of each assay and their interassay coefficients of variation are as follows:

| | Limit of sensitivity (pg) | | Interassay coefficient of variation (%) |
|---|---|---|---|
| | Lower | Upper | |
| Prolactin | 100 | 15 000 | <9 |
| GH | 60 | 6 000 | <14 |
| TSH | 1 000 | 500 000 | <12 |
| LH | 300 | 80 000 | <10 |
| FSH | 1 000 | 400 000 | <12 |

The lower limit of sensitivity is defined as the lowest amount of hormone which can be distinguished from zero (2.s.d.); the upper limit of sensitivity is taken as 2 s.d. below the amount of hormone at which the standard curve reached its plateau.

Serum samples were assayed in duplicate and samples which exceeded assay upper limits were reassayed in further dilution.

*Experimental protocol*

The protocol, drug doses and timing of intervals between injections of oestradiol valerate chosen in this investigation were identical to those used by us in a previous report (Smythe and Brandstater 1980). Oestradiol valerate (2 mg in 0·2 ml oil, subcutaneous, s.c.) was administered at monthly intervals for 3 months to 26 male Wistar rats 32 days old and weighing 85–100 g at the start of the study. Six of the rats administered oestrogen also received 100 μg bromocriptine in saline, s.c., twice weekly until 1 week before slaughter. Sesame oil (0·2 ml s.c.) was administered to 11 control rats at the same time as oestradiol was administered to test rats. All the animals were killed by decapitation 12 days after the final oestrogen injection. On the day of slaughter five rats from the oestrogen-treated population were given bromocriptine (1 mg/kg, s.c. 4 h before slaughter). Serum was collected and stored at −20°C until assayed for pituitary hormones.

*Statistics*

Data were evaluated statistically using Student's *t*-test and, in cases where a considerable scatter of data precluded this test, Wilcoxon's test for two samples was used. All results are expressed as the mean ±s.e.m.

## Results

Apart from the profound hyperprolactinaemia, chronic oestrogen treatment was found to result in significant effects on the other pituitary hormones measured in this study (see Table 1). The effects observed were in a direction opposite that on prolactin. Serum GH concentrations were significantly reduced ($P < 0{\cdot}02$) by the oestrogen treatment. The acute administration of bromocriptine abolished this effect on GH levels but chronic bromocriptine treatment had no significant effect. The effects observed on serum TSH concentration were very similar to those on GH with oestrogen treatment causing a highly significant reduction ($P < 0{\cdot}0025$) which was abolished by acute, but not chronic, bromocriptine administration. The

serum gonadotrophins, LH and FSH, were both markedly reduced ($P < 0 \cdot 01$) following the oestrogen treatment but, unlike GH and TSH, neither acute nor chronic bromocriptine administration was able to normalize the concentrations of these hormones.

## Discussion

The induction of hyperprolactinaemia following chronic high dose oestrogen administration to male rats is associated with reduced secretion of GH, TSH, LH and FSH. The results for GH and TSH are qualitatively similar to each other and paralleled the previously reported effects on hypothalamic 5-HT concentrations which occur under these circumstances (Smythe and Brandstater 1980). In that paper (following chronic oestrogen treatment) we demonstrated significant reductions in hypothalamic 5-HT concentrations which were reversed by acute but not chronic, bromocriptine. Neither DA nor NA showed this effect. These findings support

**Table 1. Serum pituitary hormone levels in chronically oestrogen-treated rats and the additional effects of bromocriptine administration**

Superscript letters indicate values significantly different from controls (unless otherwise indicated) as follows: [a] $P < 0 \cdot 005$; [b] $P < 0 \cdot 005$ (controls), $P < 0 \cdot 01$ (oestrogen alone, Wilcoxon's test); [c] n.s. (controls), $P < 0 \cdot 01$ (oestrogen alone, Wilcoxon's test); [d] $P < 0 \cdot 02$; [e] n.s. (controls or oestrogen alone); [f] $P < 0 \cdot 0025$; [g] $P < 0 \cdot 025$; [h] n.s.; [i] $P < 0 \cdot 01$; [j] $P < 0 \cdot 0025$

| Treatment | Prolactin (ng/ml) | GH (ng/ml) | TSH (ng/ml) | LH (ng/ml) | FSH (ng/ml) |
|---|---|---|---|---|---|
| Controls | $24 \pm 5$ | $97 \pm 26$ | $450 \pm 75$ | $27 \pm 6$ | $428 \pm 40$ |
| Oestrogen only | $1744 \pm 508^{a}$ | $34 \pm 4^{d}$ | $171 \pm 19^{f}$ | $3 \pm 1^{i}$ | $142 \pm 26^{j}$ |
| Oestrogen + chronic bromocriptine | $698 \pm 248^{b}$ | $39 \pm 7^{c}$ | $219 \pm 24^{d}$ | $4 \pm 1^{i}$ | $84 \pm 33^{j}$ |
| Oestrogen + acute bromocriptine | $10 \pm 5^{e}$ | $96 \pm 36^{e}$ | $252 \pm 41^{h}$ | $3 \pm 1^{i}$ | $183 \pm 33^{j}$ |

the proposed stimulatory role for 5-HT in GH secretory pathways (Smythe *et al.* 1975; Smythe 1977) and provide evidence that 5-HT may also participate in TSH secretory mechanisms. The reduced GH secretion observed in the oestrogen-treated animals is consistent with the finding of reduced pituitary content of GH in rats treated chronically with high doses of oestradiol benzoate (Daughaday *et al.* 1968).

The results of this investigation indicate that factors controlling the secretion of LH and FSH clearly differ from those which control GH and TSH and there was no parallel relationship between 5-HT responses and those of the gonadotrophins. Nor was there any apparent relationship between NA changes observed previously (Smythe and Brandstater 1980) and those changes shown here for the gonadotrophins. However, there is a relationship between gonadotrophin responses here (LH and FSH concentrations low) and those previously observed for DA. In the previous study (Smythe and Brandstater 1980) it was shown that hypothalamic DA concentrations of oestrogen-treated animals were significantly reduced but were not normalized by bromocriptine administration. Thus it is possible that a lack of functional DA activity in these hyperprolactinaemic rats may be responsible for the low gonadotrophin secretion. It is possible that a threshold concentration or activity of hypothalamic DA is required for adequate gonadotrophin releasing hormone

(GnRH) activity. There is evidence to suggest that hyperprolactinaemia is associated with a functional lack of GnRH in hyperprolactinaemic animals (McNielly *et al.* 1978; Spies *et al.* 1980) and recent evidence suggests that hyperprolactinaemia *per se* reduces pulsatile GnRH secretion from the hypothalamus (Spies *et al.* 1980). In this respect it is notable that data obtained from hypophysectomized oestrogen-treated animals as well as from intact animals indicated that the reduced hypothalamic DA activity in these hyperprolactinaemic rats is apparently due to a high degree of prolactin feedback to the brain and not (directly) to the oestrogen used to induce hyperprolactinaemia (Smythe and Brandstater 1980). The question about whether the reduced DA concentrations observed in these grossly hyperprolactinaemic rats can be interpreted as indicative of reduced functional activity of DA or, alternatively, as indicative of increased release of DA, cannot be answered with any confidence at the present time. Consequently interpretation of the present data with respect to the gonadotrophins must be treated with due caution. The role of DA in gonadotrophin secretory mechanism is controversial and in a recent review Porter *et al.* (1980) present the arguments suggesting an inhibitory role and an opposite, stimulatory role for DA in this system.

The results of this investigation confirm and extend the earlier conclusions of Meyer and Clifton (1956) concerning the chronic effects of diethylstilboestrol on pituitary hormone secretion and demonstrate that while there is gross perturbation of one hormone (prolactin), this is also associated with marked effects on the secretion of other pituitary hormones. Whether, in this case, the effects are due to a unique ability of prolactin to alter brain chemistry remains to be determined.

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