Vasoactive intestinal peptide and the stimulation of lactotroph growth by oestradiol in rats


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ABSTRACT

Treatment with a high dose of oestradiol for 6 months caused hyperprolactinaemia and pituitary hyperplasia in female Wistar–Furth rats. Changes in the vasoactive intestinal peptide (VIP) and dopamine content of the hypothalamus and pituitary were also found. The hypothalamic dopamine concentration was only slightly reduced and, although the concentration of dopamine in the pituitary was less in treated animals, the total pituitary content was increased. The concentration of VIP in the pituitary was increased by oestradiol treatment but decreased in the non-median eminence hypothalamus. In the median eminence the VIP content was increased by oestradiol treatment and the amount present correlated positively and significantly with pituitary wet weight in animals treated with both oestradiol and fluphenazine. In Fischer 344 rats, oestradiol produced greater incremental changes in pituitary wet weight and plasma concentrations of prolactin than in Wistar controls and the increase in the pituitary concentration of VIP was five times greater. Although peptide turnover has not been measured, these results suggest that oestradiol, as well as having a direct action, stimulates pituitary lactotrophs by increasing pituitary concentrations of VIP.


INTRODUCTION

The stimulatory effect of oestrogen on the growth and prolactin secretion of the anterior pituitary gland is complex. There is an undoubted direct action on pituitary lactotrophs (Nicoll & Meites, 1962; Vician, Shupnik & Gorski, 1979) but some workers have reported an inhibitory effect on dopamine secretion within the hypothalamus (Casanueva, Cocchi, Locatelli et al. 1982) and the pituitary portal blood (Cramer, Parker & Porter, 1979), as well as a reduction in the sensitivity of dopamine receptors (Raymond, Beaulieu, Labrie & Boissier, 1978). A reduction in dopamine activity, however, does not appear to be the only explanation, since oestrogen stimulates the growth of transplanted pituitaries to a lesser extent than that of glands which remain in situ (Welsch, Jenkins, Amenomori & Meites, 1971), and lesions of the median eminence in rats reduce the oestrogen stimulation of prolactin (Bishop, Kalra, Fawcett et al. 1972). There are also reports that increased prolactin-releasing activity is present in the hypothalami of rats with pituitary tumours induced by oestrogen (Nakagawa, Obara & Tashiro, 1980), and Sirbasku (1978) has produced evidence for oestrogen-induced mitogens of small molecular weight which are involved in growth-promoting effects on several tissues including the pituitary.

Vasoactive intestinal peptide (VIP) stimulates prolactin secretion in rats and man (Kato, Iwasaki, Iwasaki et al. 1978; Ottesen, Andersen, Gerstenberg et al. 1981), is present in the hypothalamus and pituitary portal blood in high concentrations (Said & Porter, 1979; Vijayan, Samson, Said & McCann, 1979), and there are VIP receptors present on pituitary membranes (Bataille, Peillon, Besson & Rosselin, 1979). For these reasons we have investigated the role of VIP in the oestrogen-mediated growth and prolactin secretion of the rat pituitary gland. We have attempted to dissociate the effects on dopamine from those on VIP by using the dopamine-blocking drug fluphenazine.

MATERIALS AND METHODS

Animals

Ten-week-old female Wistar–Furth rats were implanted s.c. with 2.5 cm lengths of silicone elastomer tubing containing 20 mg oestradiol (Sigma Chemical
Co, Poole, Dorset). In each experiment, half of the rats were also injected i.m. with 12.5 mg fluphenazine (E. R. Squibs & Sons Ltd, Hounslow, Middx) per kg once a week. Age-matched rats were used as controls.

In separate experiments treatments were continued for 70, 200 and 202 days. In a fourth experiment half the animals were examined after 144 days of treatment and half after 199 days. In all cases the plasma prolactin concentration, pituitary weight and hypothalamic VIP concentrations were measured, other measurements were made only in particular experiments as indicated in the Results section and the Tables.

Animals were killed by cervical dislocation, trunk blood was collected, and the brain and pituitary were rapidly removed. The neurohypophysis was discarded and the remaining pituitary weighed. The hypothalamus was dissected out and when the median eminence was taken separately it was removed with the aid of a microscope and forceps as described by Cuello, Horn, Mackay & Iversen (1973). All these tissues were homogenized in acetic acid (1.6 mol/l), an aliquot was taken for dopamine measurement and the remainder freeze dried. In two experiments pieces of pituitary from the control and two treatment groups were removed after weighing and fixed in 10% (v/v) formaldehyde. Wax-embedded sections were stained immunocytochemically for rat prolactin using methods described by Sternberger (1974).

VIP assay
Vasoactive intestinal peptide was measured by radioimmunoassay using the protocol, antisera and iodinated peptide obtained from Amersham International plc, Bucks. The cross-reaction with secretin, glucose-dependent insulintrophic peptide, peptide histidine isoleucine and glucagon was less than 0.05%. The sensitivity of the method was 3 pg, the intra-assay variation <5% and the interassay variation 18.5%. Tissue extracts run on a reverse-phase high-performance liquid chromatography (HPLC) column with a linear acetonitrile gradient eluted in the same position as synthetic VIP.

Catecholamine assay
Portions of acidic extracts of tissue were kept at −70 °C until assayed. Extracts were neutralized, centrifuged at 2000 g and an aliquot added to 1.0 ml 1.5 mol Tris/l, 0.125 mol EDTA/l (pH 8.6). Acid-washed alumina (10 mg) and a trace (2.9 nCi) of [3H] dopamine, to measure recovery, was added to each sample.

Extraction was carried out by mixing for 15 min at room temperature followed by three washes in dilute (0.6 mmol/l) Tris–EDTA. The mean overall recovery from this procedure was 49.3%, most of the loss occurring during absorption onto alumina; the values given have been corrected for extraction efficiency. Samples were eluted in 150 or 200 µl perchloric acid (0.1 mol/l) and 40−70 µl injected into an HPLC instrument using a Waters WISP automatic injection system. Chromatographic separation was achieved with an Altex ultrasphere reverse-phase column using a phosphate-citrate buffer at pH 4.8 containing 10% (v/v) methanol and heptane sulphonic acid (400 mg/l). Catecholamines were detected electrochemically with a ESA Coulachem 6100A detector.

Prolactin assay
Prolactin was assayed in rat plasma using the protocol and materials supplied by NIADDK, Bethesda, MD, U.S.A. Results are expressed as µg NIADDK standard PRL-RP-3/l.

Protein measurement
Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Changes in plasma prolactin, pituitary weight and histology after prolonged treatment with oestradiol
Plasma prolactin concentrations were 34 times control values after 70 days of treatment with oestradiol, increasing up to 60 times at 200 days (Table 1). The weight of the pituitary glands was increased between three and four times that of control animals (Table 1). Histology including immunocytochemistry for prolactin was performed in two experiments. At 70 days, pituitary weight was increased threefold and this was due to hyperplasia of lactotrophs without overall structural disruption or adenoma formation. Similarly, in an experiment of 200 days duration, the general appearance of the gland was still of hyperplasia rather than tumorous but some more focal areas of hyperplasia were found in some glands; such areas are considered to be precursors to adenoma formation in the rat pituitary (Berkvens, van Nesselroody & Kroes, 1980). No differences in the microscopical appearance of the glands was found between those exposed to oestrogen only and those with combined oestrogen and fluphenazine, either in the apparent number of cells staining for prolactin or in hyperplasic changes. Fluphenazine alone did not produce pituitary hyperplasia.

Pituitary dopamine
Pituitary dopamine was measured in two experiments of 199 and 202 days duration. Oestrogen-treated
pituitaries had significantly lower concentrations of dopamine per mg protein than controls but, allowing for the increased amount of tissue present, total dopamine in the pituitary was either unchanged or increased by oestrogen treatment (Table 2).

**Hypothalamic dopamine**

After 199 days of administration of oestradiol there was a small non-significant reduction in the basal dopamine content of the hypothalamus to 42±42 (S.E.M.) pmol/mg protein (n = 4) compared with 56±14 pmol/mg protein (n = 4) in controls. Dopamine turnover was not measured.

**Pituitary VIP**

After both 144 and 199 days of oestradiol treatment the concentration of VIP in the pituitary was significantly greater than that of control animals. A similar increase was found in animals treated with both fluphenazine and oestradiol (Table 2).

**Hypothalamic VIP**

In the experiment of 70 days duration and in those of 144, 199 and 202 days, the VIP concentration of the total hypothalamus was measured and expressed as a concentration (pg/mg protein). After 70 days the VIP concentration was not significantly changed but in the longer experiments the VIP concentration in the hypothalamus was reduced in animals treated with...
oestrogen and fluphenazine. In two experiments VIP was also reduced in animals treated with oestradiol only (Table 2). In the experiment of 202 days duration, where the largest changes were found, animals treated with both oestrogen and fluphenazine had significantly lower hypothalamic VIP concentrations than both controls and rats treated with oestrogen only.

In one experiment of 200 days duration, the median eminence was dissected from the remaining hypothalamus and the VIP content expressed in pg per median eminence because of the small quantity of protein present. The VIP content of this area was increased by oestradiol treatment, being 18·0 ± 6·3 pg (n = 9) in control rats and 37·0 ± 8·3 and 35 ± 6·8 pg per median eminence (n = 7 per group) in the oestradiol- and oestradiol and fluphenazine-treated groups respectively. In contrast, in the same animals VIP concentration in the rest of the hypothalamus (481 ± 58 pg/mg protein for controls) was lower in the oestrogen-treated groups (310 ± 35 when treated with oestradiol only and 384 ± 37 pg/mg protein when treated with oestradiol and fluphenazine).

Relationship of hypothalamic VIP to pituitary weight
All the values for hypothalamic VIP from the experiment of 202 days duration were plotted against the pituitary wet weight for these animals (Fig. 1). There was a positive relationship with a correlation coefficient of 0·84 (P < 0·01), indicating that although total hypothalamic VIP was reduced, those animals that retained the highest concentration also had the largest pituitaries. The same plot for the data from the experiment of 144–199 days duration, however, did not produce a significant correlation due to the presence of one animal with a low concentration of VIP in the hypothalamus and a large pituitary, although if this point were excluded the correlation (r = 0·667) was significant (P < 0·05).

In the final experiment of 200 days duration, in which the median eminence and the remaining hypothalamus were treated separately, no relation was found between pituitary weight and the concentration of VIP in the hypothalamus without the median eminence but the VIP content of the median eminence correlated significantly (r = 0·81, P < 0·05) with pituitary weight.

In no experiments where oestrogen alone was used, nor in the experiment of only 70 days duration, was any relationship found between hypothalamic VIP and pituitary wet weight.

Comparison of the effects of oestrogen on pituitary VIP in Fischer and Wistar rats
The plasma concentration of prolactin was increased 12·7 times in Fischer rats 14 days after an oestrogen
TABLE 3. Effects of 25 mg implants of oestradiol for 14 days on pituitary weight, plasma prolactin concentrations and vasoactive intestinal peptide (VIP; content of the median eminence and concentration in the anterior pituitary) in Fischer 344 and Wistar rats. Values are means ± s.e.m., n = 5 per group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pituitary weight (mg)</th>
<th>Plasma prolactin (μg/l)</th>
<th>Pituitary VIP (pg/mg protein)</th>
<th>Median eminence VIP (pg/median eminence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10·4 ± 0·5</td>
<td>37 ± 9·1</td>
<td>916 ± 427</td>
<td>24·3 ± 2·8</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>18·6 ± 2·6</td>
<td>125 ± 35</td>
<td>5037 ± 2280</td>
<td>49·4 ± 13·0</td>
</tr>
<tr>
<td>Fischer rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8·6 ± 0·2</td>
<td>11·0 ± 1·4</td>
<td>1366 ± 507</td>
<td>21·8 ± 2·8</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>18·8 ± 0·8</td>
<td>144 ± 24</td>
<td>34 679 ± 8783*</td>
<td>37·6 ± 12·0</td>
</tr>
</tbody>
</table>

*P < 0·001 compared with oestrogen-treated Wistar rats (Student's t-test).

Implant but only 3·3 times in similarly treated Wistar rats (Table 3). Although pituitary weights were similar in the two strains of rat, after oestrogen treatment, the control Fischer rats had smaller pituitaries so that the percentage increase in weight was greater in this strain. The pituitary VIP concentration was increased 5·5 times by oestradiol in the Wistar rats and 25 times that of the controls in the Fischer rats. The VIP content of the median eminence was also increased in both strains.

DISCUSSION

Plasma prolactin concentrations increased considerably as expected after the administration of high doses of oestradiol, and this stimulation of hormone secretion was accompanied by a large increase in pituitary weight with hyperplasia and later changes which may have been the start of tumour formation. While a decrease in the inhibitory influence of hypothalamic-pituitary dopamine has been considered to be a factor in the stimulatory action of oestrogen, it is unlikely to be the sole explanation. Our results revealed a non-significant decrease in hypothalamic dopamine after treatment with oestradiol for 6 months and an increase in the total dopamine content of the pituitary.

Other workers have reported stimulatory effects of oestrogen on the prolactin-releasing activity of the hypothalamus (Nakagawa et al. 1980). It was therefore of considerable interest that the concentration of the prolactin-stimulating peptide, VIP, increased greatly in the pituitary glands of animals treated with oestradiol for periods ranging from 14 to 200 days. There were regional differences in the hypothalamus since VIP increased in the median eminence and decreased in the remainder of the hypothalamus. Maletti, Rostene, Carr et al. (1982) also reported an increase in pituitary VIP and a decrease in the levels within the mediobasal hypothalamus after short-term treatment with oestradiol but these workers did not examine the median eminence separately.

It must be remembered, however, that single estimations of a peptide in the hypothalamus may not reflect its turnover at the time of sampling. VIP is present in various parts of the hypothalamus (Besson, Rotszajn, Laburthe et al. 1979; Samson, Said & McCann, 1979) and Mezey & Kiss (1985) have shown, using immunocytochemical methods, that there is a significant increase in the VIP-containing fibres of the median eminence during the physiological stimulus of lactation in the rat at a time when there is also an increase in prolactin secretion and growth of the lactotrophs.

Attempts were made therefore to correlate pituitary growth with the VIP content of the hypothalamus. Dopamine activity persisted after treatment with oestrogen, although its concentration in the pituitary was reduced and no relationship was seen between median eminence VIP and pituitary weight unless there was also dopamine blockade with fluphenazine. When this combined treatment was used there was a positive relationship between the VIP content of the median eminence and pituitary weight. When the VIP concentration in the entire hypothalamus was measured, the mean value was lower in oestrogen-treated animals but in one experiment a significant correlation was found when individual values for the VIP concentration in the hypothalamus were plotted against the pituitary weight of each animal.

This significant relationship, in two out of three experiments lasting over 100 days, does not of course prove a causative link but it is of interest that when the Wistar strain of rat, which is moderately sensitive to oestrogen, was compared with the Fischer 344 strain, which is known to be highly sensitive to the induction of pituitary hyperplasia by oestrogen (Wilkund & Gorski, 1982), the amount of VIP in the pituitary was over six times that in the Wistar rat.
Moreover this marked increase in VIP occurred at a time when there was still relatively little difference in pituitary size between the two strains. It does not seem likely, therefore, that the amount of VIP was secondarily related to the number of lactotrophs. Recently Baird, Esch, Mormede et al. (1986) have shown that fibroblast growth factor is also increased in Fischer rat pituitaries after oestadiol treatment but not in the less responsive Sprague-Dawley animals. It thus seems possible that the susceptibility of pituitary growth to oestrogen stimulation is related to the ability to induce certain peptides which may stimulate growth.

When considering the role of VIP in this respect, it has already been shown that VIP can increase intracellular calcium (Prysor-Jones, Silverlight & Jenkins, 1987), a suggested signal for mitogenic activity (Moolenaar, Tertoolen & de Laat, 1984), as well as increasing the energy metabolism of prolactin-secreting tumours (Prysor-Jones, Silverlight, Jenkins et al. 1986). It appears that, in addition to the established secretagogue action of VIP, its effect on the growth and development of lactotrophic tumours should be considered.

ACKNOWLEDGEMENTS

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REFERENCES


