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Oestrogen-induced Hyperprolactinaemia in the Rat: Reduced Concentrations of Hypothalamic Dopamine and the Effects of Bromocriptine

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Abstract

The induction of hyperprolactinaemia in the male rat following chronic oestrogen administration over 3 months was shown to result in a highly significant reduction in hypothalamic dopamine (DA) concentration as well as significant reductions in hypothalamic noradrenaline (NA) and serotonin (5-HT). Significant increases in hypothalamic DA synthesis were found 4 days after a single dose of oestrogen but not after 3 months' chronic treatment. However, after the latter treatment there was a reduction in the ratio of the level of DA to its acidic metabolites in the hypothalami of the rats.

When small doses of the ergot bromocriptine were chronically administered to oestrogen-treated rats a significant return towards normal concentrations was noted for serum prolactin and the hypothalamic catecholamines but 5-HT was suppressed even further. An acute dose of bromocriptine given to rats which had been chronically administered oestrogen caused a complete reversal of hyperprolactinaemia and a small increase in hypothalamic DA levels.

The suppressive effects of chronic oestrogen treatment on hypothalamic monoamines in intact rats was not observed in hypophysectomized rats. In hypophysectomized rats after chronic oestrogen treatment hypothalamic DA concentration was not significantly different from that of normal controls while both NA and 5-HT were significantly elevated.

In this investigation oestrogen-induced hyperprolactinaemia was found to relate to hypothalamic DA but not to NA or 5-HT. In normal and oestrogen-treated rats there was found to be a highly significant correlation between serum prolactin and hypothalamic DA concentration. The data indicate that the reduction in hypothalamic DA is primarily due to the hyperprolactinaemia and not to a direct action of oestrogen on the brain.

This study raises the possibility that reduced hypothalamic DA concentrations may be related to the degenerative hypothalamic lesions seen in chronically oestrogen-treated rats and that these lesions may be an effect of prolactin rather than oestrogen as proposed previously.

Additional keywords: 3, 4-dihydroxyphenylacetic acid, 4-hydroxy-3-methoxyphenylacetic acid, 5-hydroxyindoleacetic acid, urinary monoamine metabolites, GC/MS, selected ion monitoring, hypothalamic lesions.

Introduction

The mechanism whereby oestrogen administration induces hyperprolactinaemia and prolactin-secreting pituitary adenomas in rodents (Meyer and Clifton 1956; Caligaris and Taleisnik 1974; Lloyd *et al.* 1975) remains to be elucidated. It has been proposed that the effect of oestrogen is a direct one on the pituitary, related to reduced intracellular levels of pituitary prolactin causing an increased mitotic response (Lloyd *et al.* 1975). It has also been proposed that central serotoninergic neurones mediate the effect of oestrogen on prolactin release (Caligaris and Taleisnik 1974). Oestrogen administration depresses 'prolactin-inhibiting factor' activity in the rat hypothalamus (Ratner and Meites 1964) and since prolactin release is tonically

inhibited via hypothalamic dopamine (DA) pathways which have been shown to be subject to negative feedback by prolactin, attention has recently been directed toward the role of DA in oestrogen-induced hyperprolactinaemia (Eikenburg *et al.* 1977). Eikenburg *et al.* (1977) showed in rats that treatment for 5 days with oestrogen resulted in approximately threefold increases in serum prolactin and increased DA turnover in the median eminence but no changes were seen in DA concentrations. The study of Eikenburg *et al.* (1977) did not investigate the effects of chronic hyperprolactinaemia in the situation when prolactin-secreting tumours are present and the degree of hyperprolactinaemia is great. After chronic (3 months) treatment with oestradiol valerate in the rat prolactin-secreting pituitary tumours are seen associated with specific pathological changes in the hypothalamus (Brawer and Sonnenschein 1975; Brawer and Naftolin 1979).

The present study was undertaken in order to (i) clarify the roles of hypothalamic DA, noradrenaline (NA) and serotonin (5-HT) following chronic oestrogen-induced hyperprolactinaemia in the rat; (ii) to determine, in this situation, whether any changes in the status of hypothalamic monoamines was due to oestrogen *per se* or to prolactin feedback onto the hypothalamus; and (iii) to investigate the actions of the DA receptor stimulator, bromocriptine, in rats with chronic oestrogen-induced hyperprolactinaemia.

Materials and Methods

Drugs

The drugs employed in this investigation were the following: oestradiol valerate (Primogyn Depot, Schering Pty Ltd, Sydney), bromocriptine (CB-154, Sandoz Australia Pty Ltd, Sydney), iproniazid (Marsilid, Roche Products Pty Ltd, Sydney) and 3-iodo-L-tyrosine (Sigma Chemical Co., St. Louis, Missouri, U.S.A.).

Experimental Procedures

(*a*) *Tissue Samples*

(i) *Hypothalamic–median eminence.* In a pilot study prior to the current investigation it was ascertained that there were parallel changes in the concentrations of biogenic amines in both hypothalamic and median eminence sections of the brain of rats treated chronically with oestrogen when those sections were taken as previously described (Smythe *et al.* 1979). Therefore in this study (except where otherwise noted) it was decided to carry out biogenic amine measurements on one 'block' of tissue termed the 'hypothalamic–median eminence'. This block of tissue was dissected between levels A 3·0 mm and A 5·0 mm (Konig and Klippel 1963) and extended up from the extreme basal region approximately 1 mm. The mean wet weight of this fragment for the studies described here was $21 \cdot 8 \pm 3 \cdot 4$ (s.d.) mg, ($n = 46$).

In study 2 (described below) the hypothalamic–median eminence section was divided in two and the inferior half was described as 'median eminence' and the superior as 'hypothalamus'.

(ii) *Pituitary tissue.* In the case of all control rats it was a simple matter to dissect out the posterior lobe of the pituitaries. This was discarded and results refer to anterior pituitary glands. However, in several cases of the animals treated with oestrogen the anterior pituitary tissue had completely enveloped the posterior lobe making its dissection difficult. In these cases the posterior lobe was left intact and it was assumed that it would not have any significant effect on the results.

(*b*) *Assays*

(i) *Biogenic amines.* DA, NA and 5-HT were assayed using the quantitative mass spectrometry (selected ion monitoring, SIM) technique previously described (Smythe *et al.* 1979). The between-assay coefficient of variation for this assay was less than 4% for each amine and sensitivity was less than 1 pmole.

(ii) *Acid metabolites.* 3,4-Dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid ('homovanillic acid', HVA) and 5-hydroxyindole-3-acetic acid (5-HIAA) were assayed by the SIM technique using their pure deuterated (D_5) analogues as internal standards. The between-assay coefficient of variation for this assay was less than 5% and sensitivity less than 1 pmole for each acid.

(iii) *Rat prolactin.* Prolactin was assayed by radioimmunoassay using reagents supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, Rat Pituitary Hormone Program, Bethesda, Md. The lower limit of sensitivity of the prolactin assay was 100 pg (two s.d. from total label bound in the absence of added prolactin standard) and the upper limit was 15 ng (two s.d. below total label bound in the presence of highest prolactin standard). Serum samples were assayed in duplicate and samples which exceeded the upper limit were reassayed at a dilution of 1 in 100. The between-assay coefficient of variation was less than 9% over the range of the assay.

(*c*) *Drug Doses*

The dose and timing of intervals between injections of oestradiol valerate chosen in this investigation were the same as used by Brawer and Naftolin (1979). This protocol has been shown to cause pathological changes in the medial basal hypothalamus of rats (Brawer and Sonnenschein 1975; Brawer and Naftolin 1979) and was thus considered a likely protocol to produce changes in the status of biogenic amines in the hypothalamic–median eminence region of the brain in the present studies. Brawer *et al.* (1978) have further shown that the plasma levels of oestradiol-17β in female rats treated with 2 mg oestradiol valerate are elevated to approximately 120 pg/ml 2 weeks after the injection but plateau at 30–40 pg/ml from 4 weeks after the injection. This latter plateau level is comparable to plasma levels of oestradiol-17β in normal female rats in oestrus (Cramer *et al.* 1979).

The 'average' daily dose each oestrogen-treated animal received in these studies, calculated as oestradiol-17β, was 55 μg. This dose when administered to ovariectomized female rats produces a plasma level of oestradiol-17β which is approximately double that of intact pro-oestrous rats (Cramer *et al.* 1979). Thus in the present investigation the data of Brawer *et al.* (1978) and Cramer *et al.* (1979) would indicate that the 'average' plasma oestradiol-17β level would be approximately double that of intact rats in pro-oestrous. In order to eliminate the possibility of endogenous oestrogen being a factor in the present studies, male rats were used throughout.

Bromocriptine was administered at a dose of 100 μg per rat (approx. 1 mg/kg at start of study) twice weekly in the chronic series and at a dose level of 1 mg/kg in the acute study. This dose level is slightly less than that used by Lloyd *et al.* (1975) (3 mg/kg per day) which was shown by these workers to inhibit the proliferation of pituitary prolactin cells in rats following a high dose (12 mg) of diethylstilboestrol.

The dose of iproniazid (100 mg/kg) used in this study was intermediate between that which we have previously used to inhibit prolactin secretion in normal rats (Smythe and Brandstater 1979) and that used by Lu and Meites (1971).

The dose of 3-iodo-tyrosine (50 mg/kg) used in this study was the same as that which we have previously shown to inhibit tyrosine-3-mono-oxygenase (Smythe *et al.* 1979).

Study 1: Investigation of the Effects of Chronic Oestrogen Administration on Serum Prolactin and Hypothalamic Monoamines

Oestradiol valerate (2 mg in 0·2 ml oil) was administered subcutaneously (s.c.) 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 bromocriptine (100 μg 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 times 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 two groups of five rats from the oestrogen-treated population were given either bromocriptine (1 mg/kg, s.c., 4 h before slaughter) or the monoamine oxidase inhibitor iproniazid (100 mg/kg, intraperitoneally (i.p.), 4 h before slaughter). A further five 32-day-old male rats were administered a single dose of oestradiol valerate (2 mg in 0·2 ml oil, s.c.) and killed 1 month later together with five control animals given sesame oil in order to obtain an indication of the time course of any changes in the measured parameters. Hypothalamic–median eminence sections of the brains were rapidly dissected, homogenized in n-butanol–formic acid and assayed for DA, NA and

5-HT by SIM. Anterior pituitaries were also dissected for measurement of either pituitary hormones or biogenic amines. All results for tissues are expressed in terms of the wet weight of tissue. Serum was collected and stored at −20°C until assayed for prolactin.

Study 2: Estimation of DA Turnover Rate after Chronic (3 months) and Short-term (4 days) Oestrogen Administration

(*a*) Ten male control rats and 10 male chronic oestrogen-treated rats (3 months) were prepared in the same way as those for study 1 and were further subdivided into groups of five animals. The day prior to slaughter five controls and five oestrogen-treated rats were transferred to metabolic cages for urine collection. Forty minutes before slaughter one control group and one oestrogen-treated group were administered saline (1 ml, i.p.). The remaining two groups were administered 3-iodo-L-tyrosine (MIT, 50 mg/kg) in saline suspension (1 ml, i.p.).

(*b*) Two groups of 10 male Wistar rats 50 days old were taken. One group was administered sesame oil (0·2 ml, s.c.) and the other was administered oestradiol valerate (2 mg in 0·2 ml oil, s.c.). Four days following these single injections the animals were further subdivided into groups of five animals. These four groups were then administered saline or MIT as described in (*a*) above and were killed by decapitation 40 min later.

In this study changes in the median eminence concentrations of DA following administration of the tyrosine-3-mono-oxygenase inhibitor MIT (Smythe *et al.* 1979) were used as one measure of DA turnover. This method is similar to that of Eikenburg *et al.* (1977) who used α-methyltyrosine to inhibit tyrosine-3-mono-oxygenase. A further index of DA turnover was obtained by co-assaying DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the hypothalamus of chronic control and chronic oestrogen-treated animals. This assay was carried out using the SIM technique with appropriate deuterated standards for the acid DA metabolites (full details of the method for this assay are available on application to the author). The assay for DA metabolites was not available at the time the acute (4-day) study was carried out and was applied only to the 'chronic' animals in this study.

Study 3: Effect of Chronic Oestrogen Administration in Hypophysectomized Rats

Ten male rats of the same age were used in this study. Five of these were hypophysectomized by the transauricular route when 37 days old and then, when 120 days old, were commenced on monthly injections of oestradiol valerate (2 mg in oil, 0·2 ml, s.c.) which were continued for four injections. The five rats which were not hypophysectomized were administered sesame oil (0·2 ml, s.c.) at the same time as the hypophysectomized group received oestrogen. One of these (control) rats was later lost, leaving only four in this group. All of the rats in this study were killed by decapitation 12 days after receiving the final injection. The completeness of hypophysectomy was determined in each animal at autopsy.

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

Study 1

The effects of the various treatments on pituitary weight and serum and pituitary concentration of prolactin are shown in Table 1. Chronic treatment with oestrogen alone caused a profound and highly significant fall in hypothalamic DA concentration ($P < 0{\cdot}0005$) (Fig. 1). NA (Fig. 2) and 5-HT (Fig. 3) concentrations were also reduced but the reductions were less pronounced than that seen with DA. In this oestrogen-treated group of rats, hyperprolactinaemia was marked and the mean serum prolactin level was almost 100-fold greater than that of controls (Table 1).

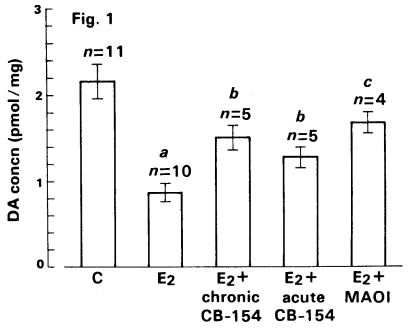
The anterior pituitary concentration of DA was reduced from $0{\cdot}8 \pm 0{\cdot}13$ pmol/mg in controls to $0{\cdot}16 \pm 0{\cdot}05$ pmol/mg in the oestrogen-treated animals.

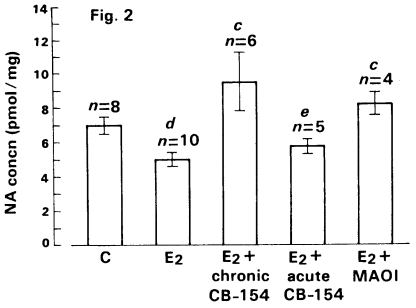
Table 1. See study 1. Anterior pituitary gland weight, serum concentration and pituitary content of prolactin in the treatment groups

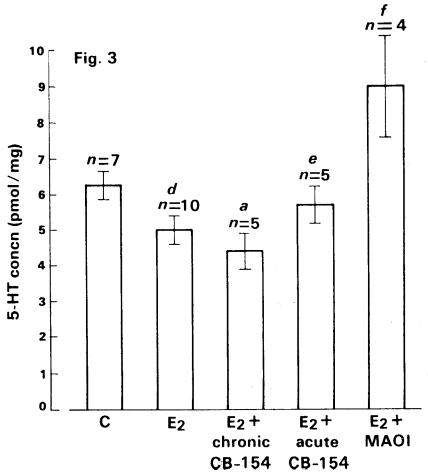
Values are means ± s.e.m. Superscripts to values indicate significance of differences from controls or other treatments as follows: *a*, $P < 0{\cdot}005$ (controls); *b*, $P < 0{\cdot}025$ (controls); *c*, $P < 0{\cdot}01$ (controls, Wilcoxon's test); *d*, $P < 0{\cdot}01$ (controls), $P < 0{\cdot}05$ (oestrogen alone, Wilcoxon's test); *e*, n.s. (controls), $P < 0{\cdot}01$ (oestrogen alone, Wilcoxon's test); *f*, $P < 0{\cdot}01$ (controls), n.s. (oestrogen alone, Wilcoxon's test). n.a., not assayed

Group	No. of animals	Pituitary weight (mg)	Serum prolactin (ng/ml)	Pituitary[A] prolactin (μg/mg)
Controls	10	$8{\cdot}0 \pm 0{\cdot}4$	$24{\cdot}2 \pm 4{\cdot}6$	$4{\cdot}8 \pm 0{\cdot}2$
Oestrogen	11	$32{\cdot}5 \pm 4{\cdot}3^a$	1743 ± 408^c	$17{\cdot}5 \pm 2{\cdot}4^a$
Oestrogen+chronic bromocriptine	6	$24{\cdot}9 \pm 5{\cdot}4^a$	698 ± 248^d	$17{\cdot}1 \pm 1{\cdot}9^b$
Oestrogen+acute bromocriptine	5	28 ± 11^b	$9{\cdot}8 \pm 5{\cdot}3^e$	n.a.
Oestrogen+acute iproniazid	5	$32{\cdot}8 \pm 10^a$	852 ± 353^f	n.a.

[A] Only six pituitaries assayed for prolactin.







Figs 1–3. Hypothalamic dopamine (DA, Fig. 1), noradrenaline (NA, Fig. 2) and serotonin (5-HT, Fig. 3) following chronic oestrogen administration (E_2) and the effects of chronic and acute bromocriptine (CB-154) and iproniazid (MAOI). Means ± s.e.m. are shown. n = number of rats in each group. Lower case letters above histograms indicate significance of differences from controls (C) or other treatments as follows: *a*, $P < 0{\cdot}0005$ (controls); *b*, $P < 0{\cdot}01$ (oestrogen alone), $P < 0{\cdot}05$ (controls); *c*, $P < 0{\cdot}01$ (oestrogen alone), n.s. (controls); *d*, $P < 0{\cdot}01$ (controls); *e*, n.s. (controls or oestrogen alone); *f*, $P < 0{\cdot}0025$ (oestrogen alone), $P < 0{\cdot}05$ (controls).

In the group of animals receiving bromocriptine chronically as well as oestrogen, hyperprolactinaemia was still evident but was significantly reduced compared with the oestrogen group ($P < 0{\cdot}05$, Table 1). As shown in Table 1 chronic bromocriptine also significantly blocked the suppression of hypothalamic DA concentration ($P < 0{\cdot}0025$) although this remained significantly lower than in the controls ($P < 0{\cdot}05$). On the other hand, in this group treated with both oestrogen and bromocriptine, NA concentrations were not significantly different from the controls while 5-HT concentrations were now highly significantly ($P < 0{\cdot}0005$) reduced.

When bromocriptine was given acutely in a single dose it caused profound and almost total inhibition of the hypersecretion of prolactin (Table 1) and also caused a small but significant ($P < 0{\cdot}025$) increase in hypothalamic DA concentration when compared with the group treated with oestrogen alone (Fig. 1). Iproniazid caused a fall in the mean serum prolactin concentration of oestrogen-treated animals but the high degree of scatter prevented this from being significant (Table 1).

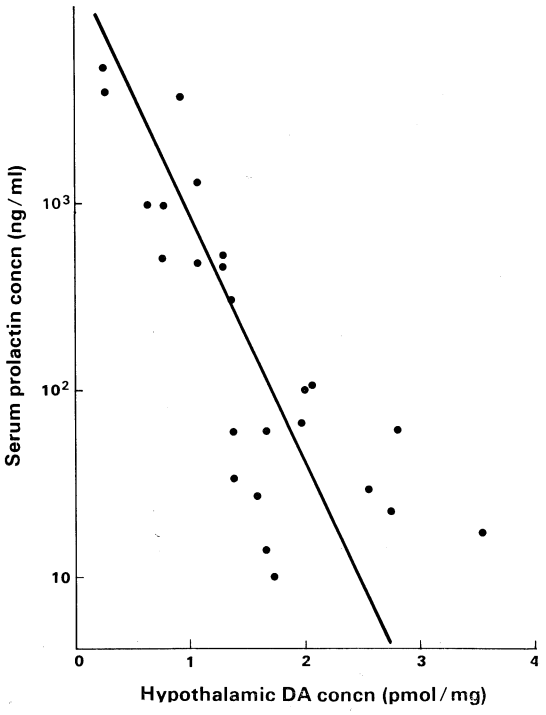


Fig. 4. Correlation between serum prolactin concentrations (y, log scale) and hypothalamic dopamine (DA) concentrations (x) for combined oestrogen-treated and control rats: $y = 3{\cdot}261–0{\cdot}329 \ln x$; $r = 0{\cdot}775$ ($P < 0{\cdot}001$).

When compared with animals given oestrogen alone, those given a single dose of iproniazid showed highly significant increases in the hypothalamic concentrations of DA ($P < 0{\cdot}0025$, Fig. 1), NA ($P < 0{\cdot}0025$, Fig. 2) and 5-HT ($P < 0{\cdot}0025$, Fig. 3). This effect of iproniazid on the hypothalamic monoamine concentrations is comparable with its effects in normal rats (Smythe and Brandstater 1979) and indicates that there are no marked changes in the rate of DA synthesis after treatment for 3 months with oestrogen (this aspect is explored further in study 2).

One month after a single injection of oestrogen the hypothalamic DA concentration ($1{\cdot}75 \pm 0{\cdot}12$ pmol/mg) was reduced ($P < 0{\cdot}06$) compared with control rats ($2{\cdot}23 \pm 0{\cdot}22$ pmol/mg) but the fall did not attain the significance of that seen at 3

months. At the same time there was a doubling of serum prolactin levels from 31·5±4·5 ng/ml (controls) to 67·6±8·8 ng/ml (oestrogen). Anterior pituitary weights were significantly greater ($P < 0·025$) 1 month after the single oestrogen injection, being increased from 7·5±0·33 mg (controls) to 13·2±1·8 mg. Neither NA nor 5-HT concentrations were significantly different in the hypothalamus of the animals 1 month after the single injection of oestrogen.

Table 2. See study 2. Serum prolactin concentrations and anterior pituitary weight in control and oestrogen-treated rats administered 3-iodo-L-tyrosine (MIT)

There were five animals per treatment group. Values are means± s.e.m. Superscripts to values indicate significance of differences from controls or other treatments as follows: *a*, $P < 0·01$ (controls, Wilcoxon's test); *b*, $P < 0·01$ (controls, Wilcoxon's test), n.s. (oestrogen+MIT); *c*, $P < 0·005$ (controls), $P < 0·0005$ (oestrogen+MIT); *d*, $P < 0·0005$ (controls +MIT, n.s. (oestrogen)

Treatment group	Weight of ant. pituitary (mg)	Serum prolactin concn (ng/ml)
Controls	10·9±0·3	37±14·6
Controls+MIT	11·7±0·5	135±19·5[c]
Oestrogen	32·0±8·5[a]	1080±750[b]
Oestrogen+MIT	30·3±2·7[a]	1009±137[d]

When the data on serum prolactin levels and hypothalamic DA concentration was examined for both controls and rats treated with oestrogen for 1–3 months (other drug treatment groups were not included) there was found to be a highly significant, inverse log–linear correlation between serum prolactin and hypothalamic DA concentration ($r = 0·775$, $P < 0·001$) which is shown in Fig. 4.

Table 3. See study 2. Effect of acute (4 days) and chronic (3 months) oestrogen administration on the turnover of rat median-eminence dopamine (DA) before and after administration of 3-iodo-L-tyrosine (MIT)

There were five animals in each treatment group. Values are means±s.e.m.

Treatment group	DA concn (pmol/mg) Before MIT	After MIT	Mean fall in DA concn after MIT (%)
Acute controls	3·1±0·3	2·3±0·4	24·5±9·7
Acute oestrogen	3·3±0·5	1·7±0·3	48·8±8·9[A]
Chronic controls	3·4±0·7	2·3±0·6	34·0±11·8
Chronic oestrogen	1·7±0·7	1·2±0·3	29·5±9·6

[A] Significantly different from acute controls ($P < 0·05$).

Study 2

This study was complicated by the chance selection of chronic oestrogen-treated animals into the two subgroups in which one subgroup showed an uneven distribution of pituitary weights. This was reflected in a similarly uneven distribution of serum prolactin levels (Table 2). No significant difference could be detected between the synthesis rate of median-eminence dopamine in oestrogen-treated rats and controls after 3 months using the MIT technique (Table 3). However, 4 days after a single

injection of oestrogen there was found a significant increase in DA clearance following MIT (Table 3). The acid metabolites of DA (DOPAC and HVA) were estimated by SIM in the hypothalami of chronic controls and chronic oestrogen-treated animals and were found to be not significantly different between the two groups (see Table 4). However, since DA concentration was reduced in the chronic oestrogen group there was a non-significant ($P < 0{\cdot}1$) increase in the ratio of concentration of DOPAC to DA and a significant ($P < 0{\cdot}005$) increase in the ratio of concentration of HVA to DA in the chronic oestrogen group as shown in Table 4. Thus there was a relative increase in DA metabolism following chronic oestrogen treatment. A lack of change

Table 4. See study 2. Hypothalamic metabolism of dopamine (DA) to 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in chronic oestrogen-treated rats

There were five animals in each treatment group. Values are means ± s.e.m. Superscripts to values indicate significant differences from controls as follows: *a*, n.s.; *b*, $P < 0{\cdot}025$; *c*, $P < 0{\cdot}005$

Treatment group	Metabolite concn (pmol/mg) DOPAC	HVA	DA	Ratio of DOPAC/DA	HVA/DA
Controls	1·1±0·1	0·67±0·05	2·0±0·2	0·54±0·04	0·33±0·03
Oestrogen	1·0±0·2[a]	0·73±0·5[a]	1·4±0·1[b]	0·74±0·1[a]	0·52±0·05[c]

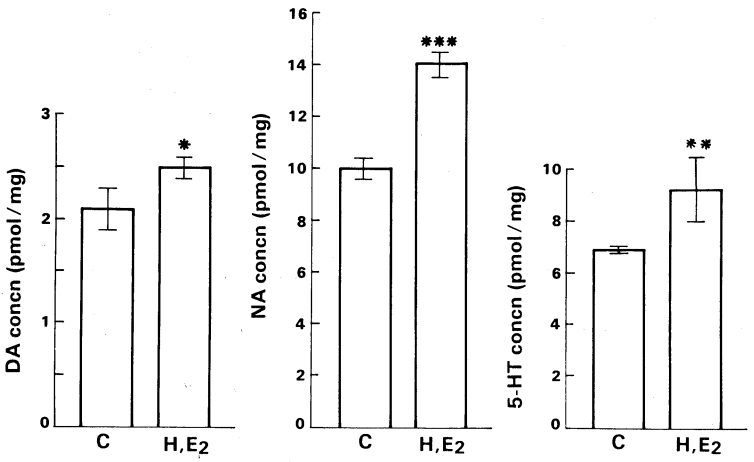


Fig. 5. Hypothalamic dopamine (DA), noradrenaline (NA) and serotonin (5-HT) concentrations in chronically oestrogen-treated hypophysectomized rats (H,E_2) compared with normal, intact controls (C). Means ± s.e.m. are shown. Significant differences from controls as follows: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

in peripheral turnover of DA or 5-HT after chronic oestrogen treatment was shown by the concentration (nmol/17 h) of urinary acidic metabolites as indicated in the following tabulation:

Treatment	HVA	DOPAC	5-HIAA
Controls	96·7±11	22·1±6·5	325±32
Oestrogen-treated	99·7± 5	20 ±4	295±27

Study 3

The effects on hypothalamic monoamines seen in intact rats after oestrogen treatment for 3 months were not observed for hypophysectomized rats. While DA concentration was not significantly different from intact, non-oestrogen-treated controls,

both NA ($P < 0{\cdot}01$) and 5-HT ($P < 0{\cdot}05$) concentrations were significantly elevated (see Fig. 5). It has since been determined that hypophysectomized rats consistently show elevated hypothalamic NA and 5-HT concentrations and unchanged DA concentrations when compared with intact controls (G. A. Smythe and J. F. Brandstater, unpublished data). Thus there is no indication of any direct oestrogenic effects on the hypothalamic monoamines in this study.

Upon inspection after decapitation one of the hypophysectomized rats used in this study was found to have a small remnant of pituitary (approx. 1 mg) in the fossa. The levels of hypothalamic DA, NA and 5-HT for this rat were in the range for normal rats and a relatively high serum prolactin was observed (107 ng/ml). This rat was considered not to have had an effective hypophysectomy and was therefore eliminated from statistical consideration.

Discussion

The data obtained in this study demonstrate that a major consequence of chronic oestrogen treatment in the rat is a reduction of hypothalamic DA concentration. Quite clearly the changes seen in hypothalamic concentrations of NA and 5-HT do not correlate with the prolactin responses and a role proposed for brain 5-HT in the oestrogen-induced release of prolactin in the rat (Caligaris and Taleisnik 1974) is not substantiated by this study.

A notable finding in this study also is the apparent ability of chronic bromocriptine to increase hypothalamic concentrations of DA and NA. This suggests that bromocriptine may exert an inhibitory effect on brain catecholamine metabolism. This observation is consistent with the findings of Hökfelt and Fuxe (1972) that bromocriptine reduces the high hypothalamic DA turnover found in animals with high prolactin secretion. The effect of bromocriptine treatment on hypothalamic 5-HT concentration was in a direction opposite to that on the catecholamines. This finding supports the contention that bromocriptine possesses antiserotonin properties which may be of importance in the interpretation of its neuroendocrine effects (Smythe 1977).

The effect of a single, acute dose of bromocriptine in almost completely inhibiting prolactin secretion in the oestrogen-treated rats is consistent with previous work demonstrating that this drug may block prolactin release directly at the level of the pituitary gland (Pasteels *et al.* 1971).

The observed highly significant correlation between serum prolactin and hypothalamic DA concentrations for normal and oestrogen-treated rats suggested that the reduction of hypothalamic DA was primarily due to hyperprolactinaemia rather than a direct brain action of oestrogen. This conclusion was substantiated by the finding in hypophysectomized rats that oestrogen treatment did not elicit any reduction in hypothalamic DA levels.

This finding is in accord with the results of Eikenburg *et al.* (1977) who showed that the increase in DA synthesis following treatment for 5 days with oestrogen was not seen in hypophysectomized rats. The data of the present study relating to DA synthesis in normal rats and rats examined 4 days after oestrogen administration is also in agreement with that of Eikenburg *et al.* (1977). However, after chronic (3 months) treatment an increased rate of DA synthesis was not detectable by the technique of tyrosine-3-mono-oxygenase inhibition. Nevertheless, the relative increase

in the metabolism of hypothalamic DA to DOPAC and HVA after chronic oestrogen treatment implies increased DA turnover but this now must be seen in terms of disappearance rather than synthesis.

The data of the present study provide a significant contribution to knowledge of the effects of continued prolactin feedback onto the hypothalamus–median eminence. Previous studies (Hökfelt and Fuxe 1972; Gudelski *et al.* 1976; Eikenburg *et al.* 1977) had indicated that prolactin feedback resulted in increased DA synthesis (for prolactin inhibition) but that steady-state DA levels remained essentially constant. This study clearly indicates that the synthetic pathway cannot maintain a continued demand for DA and hypothalamic concentrations of this monoamine are severely reduced after oestrogen treatment for 3 months in the rat. The observed change in DA status may be related to the degeneration of axons, terminals and dendrites seen in the hypothalamic arcuate nucleus of rats after 3 months treatment with oestradiol valerate (Brawer and Naftolin 1979). Furthermore, the present investigation suggests that the lesions described by Brawer and Naftolin (1979) and proposed by these workers to be a direct oestrogenic effect may, in fact, be prolactin-induced. It is notable, as pointed out by Brawer and Naftolin (1979), that the action of oestrogen in modifying hypophysiotrophic circuitry in the adult rat differs markedly from its sexual differentiation action in neonatal animals and it is possible that only the latter effect is due to oestrogen *per se*.

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