Loss of Hypothalamic Dopaminergic Control of Prolactin Secretion in the Hyperprolactinaemic Rat

G. A. Smythe, J. E. Bradshaw and M. Duncan

Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst, N.S.W. 2010.

Abstract

The possibility that chronic hyperprolactinaemia results in loss of the ability of hypothalamic dopamine activity to inhibit prolactin secretion was studied in rats. Two degrees of hyperprolactinaemia (moderate and gross) were induced in the animals following the chronic administration of two different doses of oestradiol valerate. In rats with high chronic serum prolactin concentrations (approximately 20 times normal) there was a profound increase in prolactin secretion following inhibition of brain dopamine (DA) synthesis by 3-iodo-L-tyrosine, indicating intact and highly active hypothalamic DA-inhibitory control of prolactin release. However, the degree of hypothalamic inhibition of prolactin release relative to normal controls was significantly reduced. In animals with grossly elevated chronic serum prolactin concentrations (approximately 100 times normal) a prolactin response to DA synthesis inhibition was absent despite a highly significant reduction in hypothalamic DA concentrations induced by 3-iodo-L-tyrosine. These observations show that chronic and gross hyperprolactinaemia in the rat results in loss of hypothalamic DA inhibitory control of prolactin secretion. The use of 3-iodo-L-tyrosine to block brain DA synthesis in these studies has provided significant new data relating to prolactin control in hyperprolactinaemic states and indicates that this compound could be a useful clinical tool in the study of human hyperprolactinaemia.

Introduction

It is well established that prolactin can act in a negative feedback loop to increase the activity of tuberoinfundibular dopaminergic (TIDA) neurons of the hypothalamus–median eminence (Hökfelt and Fuxe 1972; Gudelsky *et al.* 1976; Gudelsky and Porter 1980). In the short term, at least, the administration of exogenous prolactin or the induction of high levels of prolactin secretion in the rat result in increased dopamine (DA) turnover in the median eminence without altering steady-state concentrations of DA (Gudelsky *et al.* 1976; Eikenburg *et al.* 1977). When circulating prolactin levels are raised in the rat 3–5 days following oestrogen (E_2) administration there is a significant increase in median eminence DA turnover and an increased prolactin release from the pituitary in response to tyrosine hydroxylase inhibition with α-methyl-tyrosine (Eikenburg *et al.* 1977). However, we have recently presented evidence that this initial response to increased prolactin secretion is not maintained following chronic hyperprolactinaemia induced by E_2 treatment in the rat (Smythe and Brandstater 1980). After treatment for 3 months with E_2, when anterior pituitary weight is increased fourfold and serum prolactin is increased nearly 100-fold, median eminence DA concentrations are highly significantly reduced and there is no change in DA turnover detectable following tyrosine hydroxylase inhibition (Smythe and Brandstater 1980). Furthermore, this latter study indicated that there was a lack of

prolactin response to inhibition of brain DA synthesis in these animals. These findings suggest the possibility that in the presence of pituitary tumors and marked hyper-prolactinaemia there is reduced hypothalamic DA inhibitory control of prolactin secretion in the rat when, indeed, it would be anticipated that such inhibitory activity should be high due to the negative feedback function of prolactin itself. It is notable that such a situation with loss of hypothalamic DA inhibition of prolactin secretion seems to occur in humans with prolactin-secreting tumors (Fine and Frohman 1978).

The aim of the present study was to investigate the relationship between hypothalamic DA activity and the degree of hyperprolactinaemia induced by various doses of E_2 in the rat.

Materials and Methods

Experimental Animals

Immature male rats of the Wistar strain, approximately 30 days old and weighing 80–100 g at the commencement of the study, were used. The animals were housed in a room in which the ambient temperature varied from 18 to 22°C with a lighting schedule that provided 12-h dark–light cycles. Food and water were available *ad libitum*.

Experimental Procedures

In this investigation two degrees of hyperprolactinaemia were induced in the animals by the administration of oestradiol valerate according to two different protocols that varied with respect to dosage and timing. In one protocol oestradiol valerate (50 μg in 0·2 ml sesame oil, subcutaneous, s.c.) was administered in six injections 1 week apart and in the other a larger dose of oestradiol valerate (1 mg in 0·2 ml sesame oil, s.c.) was administered in six injections 2 weeks apart. Control animals for each protocol received sesame oil (0·2 ml, s.c.) only.

Ten days after the final E_2 injection in each series (day of slaughter) each control and E_2-treated group was divided into two subgroups, one of which was administered saline (1 ml, intraperitoneal, i.p.) and the other was administered 3-iodo-L-tyrosine (MIT, 50 mg/kg, i.p.). The animals were killed by decapitation 30 min after their saline or MIT injection and trunk blood was collected. Where applicable, the brains were rapidly removed and the medial–basal hypothalamus was dissected out as described below. Anterior pituitary glands were dissected out and weighed immediately. Because interpretation of earlier studies was complicated by a wide range in response to E_2 indicated by a spread of pituitary weights in rats receiving high doses of E_2 (Smythe and Brandstater 1980), in the present investigation a larger number of animals than was required was treated with high dose E_2 and only those (13 out of 17) with anterior pituitary weights in the range 20–50 mg were used. Other animals having heavier or lighter pituitaries, with the one exception noted later, were discarded.

MIT was used in these studies as a tyrosine hydroxylase inhibitor because it is devoid of toxic side-effects and has rapid and relatively specific ability to inhibit CNS DA synthesis (Smythe *et al.* 1974, 1979). The dose of MIT used in these studies was the same as that which we have previously shown to result in a highly significant reduction in hypothalamic DA synthesis within 30 min (Smythe *et al.* 1979).

Assays

The serum level of prolactin of each rat was assayed by radioimmunoassay using reagents supplied by the National Institute of Arthritis, Metabolic and Digestive Diseases, Rat Pituitary Hormone Program, Bethesda, Maryland. The lower limit of sensitivity of the prolactin assay was 100 pg (two standard deviations from total label bound in absence of added prolactin standard) and the upper limit was 20 ng (two standard deviations below total label bound in presence of highest prolactin standard). Serum samples were assayed in duplicate and samples that exceeded the upper limit were reassayed in dilution. The between-assay coefficient of variation was less than 9% over the range of the assay.

DA was assayed by a specific computerized GC/MS selected-ion monitoring (SIM) procedure as previously described (Smythe *et al.* 1979). The between-assay coefficient of variation was less than 3% and sensitivity was less than 1 pmol. Results are expressed in picomoles per milligram (wet weight) of tissue. The hypothalamic samples were rapidly dissected as previously described (Smythe *et al.* 1979; Smythe and Brandstater 1980). The tissue block was approximately a 2-mm cube in order to ensure that the full extent of the arcuate nucleus and median eminence was obtained (Monroe *et al.* 1972; Amback and Palkovits 1979) and the mean wet weight of the samples was $11 \cdot 5 \pm 0 \cdot 5$ (s.e.) mg.

Drugs

The drugs employed in this investigation were the following: oestradiol valerate (Primogyn Depot), Schering Pty Ltd, Sydney, and 3-iodo-L-tyrosine (monoiodotyrosine, MIT), Sigma Chemical Co., St Louis, Mo., U.S.A.

Statistics

Data were evaluated statistically using Student's *t*-test and Wilcoxon's test for two samples.

Table 1. Anterior pituitary weights and serum prolactin concentrations in rats with two degrees of hyperprolactinaemia and the effects of brain tyrosine hydroxylase inhibition by 3-iodo-L-tyrosine (MIT)
MIT given at the rate of 50 mg/kg, i.p. Means$\pm$s.e.m. are shown. Superscripts: a, $P < 0 \cdot 0025$ *v.* group A; b, $P < 0 \cdot 01$ *v.* group A; c, $P < 0 \cdot 0025$ *v.* group A and *v.* group D; d, $P < 0 \cdot 0025$ *v.* group E: not significantly different from group B; e, $P < 0 \cdot 0025$ *v.* group E, $P < 0 \cdot 05$ *v.* group C; f, $P < 0 \cdot 0025$ *v.* group C, not significantly different from group H; g, $P < 0 \cdot 05$ *v.* group B

Treatment group	No. in group	Wt of anterior pituitary (mg)	Serum prolactin concn (μg/l)	Relative dopamine inhibition
I. Low dose E_2				
A. Controls+saline	5	$9 \cdot 3 \pm 0 \cdot 5$	$18 \cdot 4 \pm 1 \cdot 4$	—
B. Controls+MIT	6	$8 \cdot 5 \pm 0 \cdot 8$	102 ± 15^b	$79 \cdot 3 \pm 3 \cdot 2$
C. E_2+saline	6	$27 \pm 2 \cdot 4^a$	380 ± 52^c	—
D. E_2+MIT	6	$27 \pm 3 \cdot 7$	1210 ± 180	$67 \pm 4 \cdot 3^g$
II. High dose E_2				
E. Controls+saline	5	$9 \cdot 9 \pm 0 \cdot 3$	$21 \cdot 5 \pm 5 \cdot 7$	—
F. Controls+MIT	5	$10 \cdot 7 \pm 0 \cdot 6$	$132 \cdot 8 \pm 20^d$	$82 \cdot 2 \pm 2 \cdot 4$
G. High dose E_2+saline	7	$37 \cdot 8 \pm 3 \cdot 5^e$	2525 ± 520^f	—
H. High E_2+MIT	6	$35 \cdot 7 \pm 2 \cdot 6$	2366 ± 780	0

Results

The two protocols used for E_2 administration in this investigation produced two different degrees of hyperprolactinaemia in the rats. Table 1 shows the pituitary weights and serum prolactin levels for the various groups. The low dose E_2 protocol resulted in animals with serum prolactin concentrations highly significantly greater ($P < 0 \cdot 0005$) than controls (an approximate increase of 20-fold). A further highly significant increase ($P < 0 \cdot 0025$) in serum prolactin level was achieved with the high dose E_2 protocol. In this case serum prolactin concentrations were approximately 100-fold greater than controls. Anterior pituitary weights were significantly raised ($P < 0 \cdot 0025$) by the low dose E_2 protocol and were elevated even higher ($P < 0 \cdot 05$) by the high dose E_2 protocol. Inhibition of brain DA synthesis by MIT caused a

highly significant ($P < 0\cdot01$) increase in serum prolactin levels in the control group and in the low dose E_2 group ($P < 0\cdot0025$) when compared with their respective controls but had no significant effect on the serum prolactin levels of the group of animals on high dose E_2. In order to be able to relate the responses of the hyper-prolactinaemic animals to the responses of controls following MIT administration, the relative degree to which dopaminergic tuberoinfundibular mechanisms were inhibiting prolactin release (called relative dopamine inhibition, I) was calculated using the following formula:

$$I = (S - B)/S,$$

where S is individual stimulated prolactin level after MIT in μg/l serum; B is mean basal serum prolactin concentration (μg/l) for control (saline-treated) rats. Percentage relative dopamine inhibition for the various groups is shown in Table 1 and was found to be significantly reduced ($P < 0\cdot05$) in the low dose E_2 treatment group and zero ($-12\cdot1\%$) in the high dose E_2 treatment group. In order to verify that DA synthesis had indeed been inhibited in the high dose, non-responsive E_2 group after MIT, hypothalamic DA concentrations were measured. As shown in Fig. 1 a significant
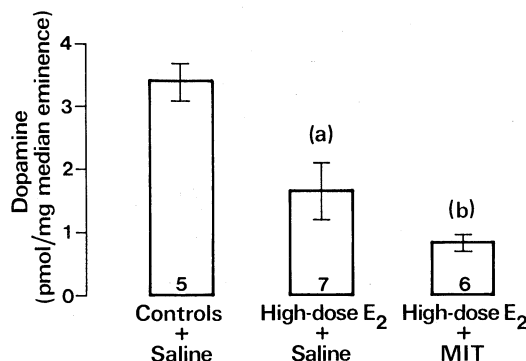

Fig. 1. Hypothalamic dopamine concentrations in control rats (chronic sesame oil) 30 min after saline, in high dose E_2-treated rats (1 mg oestradiol valerate each 2 weeks for 10 weeks) 30 min after saline and in high dose E_2-treated rats 30 min after 3-iodo-L-tyrosine (MIT, 50 mg/kg). Means±s.e.m. are shown. Numbers at the foot of each histogram indicate the number of animals in each group. (a) $P < 0\cdot01$ v. saline controls; (b) $P < 0\cdot05$ v. E_2–saline group.

reduction ($P < 0\cdot05$) of hypothalamic DA concentrations from already very low levels was caused by the administration of MIT. The fall to approximately 50% of the levels of the saline controls is similar to the changes in the hypothalamus of normal control rats after MIT (Smythe et al. 1979) but, due to the lower basal level, is indicative of a lower turnover rate for hypothalamic DA in the E_2-treated rats as noted previously (Smythe and Brandstater 1980). It is notable that one of the animals excluded from the study due to an out of range anterior pituitary gland weight (105 mg) was examined further and found to have a concentration of DA in the hypothalamus of $0\cdot2$ pmol/mg which is the lowest we have ever recorded. This was associated with a serum prolactin concentration of approximately 9 mg/l.

Discussion

The results of this investigation demonstrate that there is reduced hypothalamic DA inhibitory control of prolactin secretion in the rat following chronic hyperprolactinaemia induced by E_2. The degree to which this inhibitory control is lost is related to the basal serum prolactin concentration and the loss seems total when the anterior pituitary weight reaches proportions of about four times normal or serum prolactin concentration of the order of 100 times normal. Under these circumstances hypothalamic DA turnover and concentrations are less than 50% of normal but it is not clear to what extent the residual DA represents that which would normally be involved in prolactin-inhibitory mechanisms.

We have previously demonstrated that there is a highly significant inverse correlation between serum prolactin concentration and hypothalamic DA concentrations in rats rendered hyperprolactinaemic by chronic E_2 administration (Smythe and Brandstater 1980). Furthermore, it is important to emphasize that the effects on brain DA were concluded to be due to hyperprolactinaemia and not to a direct brain action of E_2. This conclusion was substantiated by the lack of effect of chronic E_2 administration on hypothalamic DA concentrations in hypophysectomized rats (Smythe and Brandstater 1980). A basic premise of the present investigation is that the extent to which hypothalamic dopaminergic mechanisms contribute to the tonic inhibition of prolactin release can be revealed by blockade of central DA synthesis. Following DA synthesis blockade, the relative change in prolactin release over basal secretion would thus provide a measure of the degree to which dopaminergic mechanisms are keeping prolactin release in check. On this basis, the data of the present study indicate that central dopaminergic pathways are contributing to the inhibition of some 80% of the releasable prolactin in control animals. The degree of dopaminergic inhibition of prolactin is significantly reduced to 67% in the chronic low dose E_2-treated rats and is reduced to zero in the grossly hyperprolactinaemic animals (high dose E_2 group). Since it was shown that the MIT was indeed effective in blocking DA synthesis, in this latter case there can be no conclusion other than that there is no hypothalamic DA inhibition of prolactin release in the grossly hyperprolactinaemic group of rats. This is not to say there is no hypothalamic DA activity *per se* but rather that there is a loss of 'connection' between any such activity and prolactin inhibitory mechanisms.

The reasons for the apparent loss of hypothalamic DA inhibitory control over prolactin release remain obscure at this time. The possibility that DA inhibitory control is blocked due to a direct pituitary effect of the administered E_2 (Raymond et al. 1978) must be considered but is unlikely for the following reasons: (1) the animals were killed 10 days after the final E_2 injection and it is doubtful that the circulating E_2 concentrations would reach the levels necessary for even partial blockade (10^{-9} M; Raymond et al. 1978); (2) anterior pituitary glands taken from male rats treated with E_2 according to the high dose protocol used in this study are at least as sensitive to *in vitro* DA inhibition of prolactin release as pituitaries from control animals (G. A. Smythe and P. N. Gonski, unpublished observation); and (3) a single dose of bromocriptine (100 μg) totally suppresses serum prolactin release in chronic high dose E_2-treated rats (Smythe and Brandstater 1980). It seems more likely that chronic gross hyperprolactinaemia either induces changes in (i) specific

prolactin inhibiting factor (PIF) neurons so that these no longer respond to hypo-
thalamic neuronal activity; or (ii) DA pathways to the pituitary. As previously
suggested (Smythe and Brandstater 1980) these changes may be degenerative and be
related to the neuronal lesions seen in the hypothalamic arcuate nucleus of rats after
chronic E_2 treatment (Brawer and Naftolin 1979). The loss of hypothalamic DA
inhibitory control of prolactin secretion in the hyperprolactinaemic rat demonstrated
in this investigation is in accord with similar findings in patients with prolactin-
secreting tumors (Fine and Frohman 1978; Van Loon 1978).

When taken with the data from other investigations using acute E_2 administration,
the present investigation suggests the following course of events when E_2 treatment
is initiated and maintained: E_2 stimulates lactotroph hyperplasia and increased
prolactin release which results in the activation of the negative feedback system of
the hypothalamus. Initially (after approximately 3 days) this results in the maintenance
of basal serum prolactin levels near normal but there is an enhanced prolactin response
to DA synthesis inhibition relative to controls (Eikenburg·et al. 1977). However, as
the E_2 stimulus and lactotroph hyperplasia maintain the drive to release more
prolactin, we suggest that a limit to hypothalamic DA activity is reached after which
serum prolactin levels cannot be kept near normal. As long as such a maximum
level of hypothalamic DA activity is maintained and the circulating prolactin concen-
tration continues to rise, then clearly the relative response to DA synthesis inhibition
would decrease (e.g. low dose E_2 group in this investigation). Eventually it appears
that the elevated hypothalamic DA activity (turnover) is not maintained in the presence
of chronic gross hyperprolactinaemia and a stage is reached when there is a total loss
of hypothalamic DA control of prolactin release with consequent autonomous
pituitary prolactin secretion (high dose E_2 group).

If the above hypothesis is true then it would mean that the basal prolactin secretion
rate and the prolactin response following DA synthesis blockade taken together
would provide an excellent means of estimating the extent of hypothalamic DA
control of prolactin release in various states of hyperprolactinaemia. This has
important clinical implications in investigating human hyperprolactinaemia and for
the detection of autonomous prolactin-secreting pituitary adenomas. The absence
of toxic side-effects and rapid clearance of MIT in human subjects (Smythe et al.
1975) suggest this as the tool of choice for brain DA synthesis inhibition in such
studies and our preliminary clinical findings (Smythe et al. 1982) support this proposal.

References

Ambach, G., and Palkovits, M. (1979). The blood supply of the hypothalamus in the rat. In 'Anatomy
    of the Hypothalamus'. (Eds P. J. Morgane and J. Panksepp.) pp. 267–377. (Dekker: New York.)
Brawer, J. R., and Naftolin, F. (1979). The effects of oestrogen on hypothalamic tissue. In 'Sex
    Hormones and Behaviour'. [Ciba Foundation Symposium, **62**, 19–33.] (Excerpta Medica:
    Amsterdam.)
Cramer, O. M., Parker, C. R., and Porter, J. C. (1979). Estrogen inhibition of dopamine release into
    hypophysial portal blood. *Endocrinology* **104**, 419–22.
Eikenburg, D. C., Ravitz, A. J., Gudelsky, G. A., and Moore, K. E. (1977). Effects of estrogen on
    prolactin and tuberoinfundibular dopaminergic neurons. *J. Neural Transm.* **40**, 235–44.
Fine, S. A., and Frohman, L. A. (1978). Loss of central nervous system component of dopaminergic
    inhibition of prolactin secretion in patients with prolactin secreting pituitary tumours. *J. Clin.
    Invest.* **61**, 973–80.

Gudelsky, G. A., Simpkins, J., Mueller, G. P., Meites, J., and Moore, K. E. (1976). Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. *Neuroendocrinology* **22**, 206–15.

Gudelsky, G. A., and Porter, J. C. (1980). Release of dopamine from tuberoinfundibular neurons into pituitary stalk blood after prolactin or haloperidol administration. *Endocrinology* **106**, 526–9.

Hökfelt, T., and Fuxe, K. (1972). Effects of prolactin and ergot alkaloids on the tuberoinfundibular dopamine (DA) neurons. *Neuroendocrinology* **9**, 100–22.

Monroe, B. G., Newman, B. L., and Shapiro, S. (1972). Ultrastructure of the median eminence of neonatal and adult rats. In 'Brain–Endocrine Interaction. Median Eminence: Structure and Function'. (Eds K. M. Knigge, D. E. Scott and A. Weindl.) pp. 7–26. (Karger: Basel.)

Raymond, V., Beaulieu, M., Labrie, F., and Boissier, J. (1978). Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. *Science* **200**, 1173–5.

Smythe, G. A., and Brandstater, J. F. (1980). Oestrogen-induced hyperprolactinaemia in the rat: reduced concentrations of hypothalamic dopamine and the effects of bromocriptine. *Aust. J. Biol. Sci.* **33**, 329–39.

Smythe, G. A., Brandstater, J. F., and Lazarus, L. (1974). Rapid induction of prolactin secretion by 3-iodo-L-tyrosine. *Neuroendocrinology* **14**, 362–4.

Smythe, G. A., Brandstater, J. F., Bradshaw, J. E., and Lazarus, L. (1979). A simple procedure for the assay of brain biogenic amines by selected-ion monitoring: Its application to the elucidation of the mechanism of prolactin release induced by 3-iodo-L-tyrosine. *Aust. J. Biol. Sci.* **32**, 335–41.

Smythe, G. A., Compton, P. J., and Lazarus, L. (1975). The stimulation of human prolactin secretion by 3-iodo-L-tyrosine. *J. Clin. Endocrinol. Metab.* **40**, 714–16.

Smythe, G. A., Ho, K., and Lazarus, L. (1982). Prolactin levels and the response to central dopamine synthesis blockade in woman on oral contraceptives. *IRCS Med. Sci.* **10**, 124.

Van Loon, G. R. (1978). A defect in catecholamine neurons in patients with prolactin-secreting pituitary adenoma. *Lancet* **ii**, 868–71.