Characterization of the inhibitory effects of hyperprolactinaemia on the mechanism controlling LH secretion in chronically ovariectomized rats

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Summary. The time-course of the inhibitory effect of hyperprolactinaemia on LH secretion was delineated. Hyperprolactinaemia was induced in ovariectomized rats with injections of domperidone or ovine prolactin and circulating LH levels were measured from 1 h to 9 days after the treatment. Inhibition of LH secretion occurred within 2–4 h after treatment, and was maintained (provided that serum prolactin remained elevated) for a period of 6 days only. Thereafter LH levels increased to become insignificantly different from control levels on Day 9. A reduction in pituitary responsiveness was not associated with the acute or sub-chronic inhibition of LH secretion, although a significant fall in responsiveness was observed simultaneously with the return of serum LH levels to control values. No changes in hypothalamic LH-RH content was found. It is concluded that an impairment of pituitary function is not responsible for the inhibitory action of prolactin on LH secretion.

Introduction

The causal relationship between elevated circulating prolactin concentrations and suppressed LH secretion observed in many amenorrhoeic women (McNeilly, 1979) has also been demonstrated in laboratory rats following artificial induction of hyperprolactinaemia (McNeilly, 1980). Ovariectomized female rats have been used to study the central anti-gonadotrophin effects of prolactin because the marked inhibition of serum LH levels in the absence of the ovaries indicates that prolactin can act directly on the hypothalamic/pituitary axis (Beck, Engelbart, Gelato & Wuttke, 1977; Grandison *et al.*, 1977; Vasquez, Nazian & Mahesh, 1980; Carter & Whitehead, 1981a).

Investigations of the mechanism(s) by which prolactin exerts its central effects have suggested that the inhibition of LH secretion occurs via a blockade of release of hypothalamic LH-RH and that an attenuation of pituitary responsiveness to LH-RH is not involved (Gudelsky, Simpkins, Mueller, Meites & Moore, 1976; Grandison *et al.*, 1977; Gil-Ad *et al.*, 1978; Vasquez *et al.*, 1980; Carter & Whitehead, 1981b). However, this conclusion should be treated with caution since previous studies have involved only isolated determinations of pituitary responsiveness or LH-RH release, whereas the inhibition of LH secretion during hyperprolactinaemia in ovariectomized rats has been shown to be a dynamic process with an acute onset (Gudelsky *et al.*, 1976; Flint & Ensor, 1981), but limited duration (Beck & Wuttke, 1977; Grandison *et al.*, 1977), circulating LH concentrations gradually returning to control values despite maintained hyperprolactinaemia. Therefore, a role for reduced pituitary responsiveness during the inhibitory process has not been ruled out. It is

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clear that before investigations into the precise nature of the inhibitory mechanism are undertaken, a careful characterization should be made of hypothalamic and pituitary function throughout the period of inhibition. We have therefore investigated the acute and chronic effects of induced hyperprolactinaemia on LH secretion, pituitary responsiveness to LH-RH and hypothalamic LH-RH content.

Materials and Methods

Animals. Female rats of the Porton-Wistar strain weighing between 200 and 250 g were maintained under conditions of controlled lighting (lights on 06:00–18:00 h) and constant temperature (23°C). Food and water were available *ad libitum*. On a random day of the oestrous cycle rats were ovariectomized using standard procedures. They were used for experimentation 3 and 5 weeks after ovariectomy.

Blood sampling, drug treatment and radioimmunoassays. Blood samples were taken via cardiac puncture under light ether anaesthesia. Whole blood was allowed to clot, the cells were separated by centrifugation, and the serum was stored at –20°C. Domperidone (4 mg/kg; Janssen Pharmaceuticals, Marlow, U.K.) dissolved in ethanol-saline (1:20 v/v) and ovine prolactin (4 mg/kg; Sigma, Poole, U.K.) dissolved in 0.01 M-NaHCO₃ were administered subcutaneously. Control rats received injections of the appropriate vehicle.

Serum and perfusate LH concentrations and serum concentrations of prolactin were measured by radioimmunoassays using the procedures outlined by the NIH, and are expressed in terms of NIAMDD-rat-LH-RP1 and NIAMDD-rat-PRL-RP2, the sensitivities being 10 ng/ml and 1 ng/ml respectively. The inter- and intra-assay variations were calculated to be, respectively, 10.0 and 9.0% for LH and 10.3 and 8.7% for prolactin. The concentration of LH-RH in hypothalamic extracts was assayed by a single-antibody radioimmunoassay (Jeffcoate, Fraser, Holland & Gunn, 1974) using synthetic LH-RH (Hoechst, Frankfurt, West Germany) and LH-RH antiserum supplied by Dr H. M. Fraser (M.R.C. Unit of Reproductive Biology, Edinburgh, U.K.). The inter- and intra-assay variations were calculated to be 14.2 and 8.3% respectively.

Measurement of pituitary responses to LH-RH. An *in-vivo* measure of pituitary responsiveness to LH-RH was obtained by administering a single bolus of LH-RH (100 ng in 0.1 ml 0.9% (w/v) NaCl) via cardiac puncture into rats under light ether anaesthesia. Pre- and post-stimulation blood samples were taken at the times shown in the 'Results' and serum LH values were determined. *In-vitro* determinations of pituitary LH responses to LH-RH were performed using the perfusion system described by Carter & Whitehead (1981c). Isolated hemi-pituitary glands were perfused at a rate of 0.2 ml/min with Krebs-Ringer bicarbonate, containing bovine serum albumin (2.5 g/l) and glucose (2 g/l). Perfusate fractions were collected every 10 min and the glands were challenged with two 5-min pulses of LH-RH (10 ng/ml) which were separated by an interval of 1 h.

LH-RH content of the medial-basal hypothalamus. Whole brains were excised and placed in ice. The medial-basal hypothalamus (MBH) consisting of a block of tissue approximately 1 mm in all planes around the median eminence (mean $\pm$ s.e.m. weight, 9.2 $\pm$ 1.8 mg), was dissected out within 2 min of killing. The tissue was weighed immediately and the LH-RH extracted according to the rapid method described by Koch & Baram (1976). Supernatants obtained after the extraction procedure were snap frozen and stored at –20°C until assayed for LH-RH concentration.

Experimental protocol. Chronically ovariectomized rats were given a single injection of domperidone or ovine prolactin and serum levels of LH and prolactin in frequent samples of blood were measured. In two other groups of rats similarly treated, pituitary responsiveness to LH-RH was determined *in vivo* 4 h after treatment with domperidone or ovine prolactin.

In the second series of experiments chronically ovariectomized rats were injected with domperidone twice daily for 8 days and once only on Day 9 at the times as shown in the 'Results'. Blood samples were taken at 09:00 h on Day 1 and daily at 14:30 h, and serum levels of LH and

prolactin were measured. Other groups of rats were treated similarly for different numbers of days, as shown in the 'Results', before being killed at 11:00 h on the final day of treatment. Pituitary glands were taken for in-vitro determinations of responsiveness to LH-RH and MBH blocks were taken for determinations of LH-RH content.

Analysis of results. All the results are expressed as the mean $\pm$ s.e.m. When the data in two or more control groups were not statistically significantly different, only one group is shown in the figures for the sake of clarity. The pituitary responsiveness to LH-RH in the in-vitro determinations was taken to be the difference in mean LH concentration between the peak value directly following the pulse, and the value preceding the peak. The statistical significance of differences between hormone levels or responses within groups, or between individual control and treated groups, was determined by a Student's *t* test. Differences between multiple groups were analysed by one-way Gaussian ANOVA, followed by post-hoc testing using the Newman-Keuls test. The level of significance chosen was $P < 0.05$.

Results

Experiment 1: effect of acute hyperprolactinaemia on serum levels of LH and prolactin, and pituitary responsiveness to LH-RH in vivo

Acute s.c. treatment with domperidone or ovine prolactin did not modify the concentration of serum LH in blood samples taken 1 or 2 h after the injections compared with vehicle-treated controls (Text-fig. 1a). However, after 4 h the levels of LH in both treated groups were significantly reduced, and significantly lower than the 4-h control values. The inhibition of LH secretion was maintained at 6, 8 and 16 h after treatment, but at 24 h there was no significant difference between the control and treated groups. Serum prolactin was markedly raised 1 h after domperidone treat-

Text-fig. 1. Effect of a single s.c. injection at time 0 of domperidone (○, 4 mg/kg, N = 5) or ovine prolactin (□, 4 mg/kg, N = 4) on mean $\pm$ s.e.m. serum levels of (a) LH and (b) prolactin in chronically ovariectomized rats. Vehicle-treated controls are also shown (●, N = 6). † $P < 0.05$ compared to previous value (paired *t* test). * $P < 0.05$ compared to equivalent control value (Newman-Keuls test).

ment and significantly elevated prolactin levels were maintained until 16 h, although it appeared that circulating prolactin levels were returning to control values 8–24 h after treatment (Text-fig. 1b). No changes in endogenous serum prolactin levels were observed in the control or prolactin treated animals throughout the experiment. Since ovine prolactin does not significantly cross-react with the antiserum for rat-prolactin, the raised levels of prolactin in these animals would not have been detected in the radioimmunoassay.

Acute hyperprolactinaemia, induced with s.c. injections of domperidone or ovine prolactin, did not modify the in-vivo LH response to a bolus injection of LH-RH (Text-fig. 2). Although the initial treatment resulted in a significant inhibition of serum LH in both groups, and the absolute heights of the LH-RH induced surges of LH were uniformly lower in the treated animals, the increment of LH secretion in response to LH-RH was similar in all the groups. Serum prolactin levels were raised after domperidone treatment, but no changes in the prolactin concentrations were observed after the LH-RH stimulus (Text-fig. 2).

Text-fig. 2. Effect of pre-treatment at 12:00 h with (b) domperidone (4 mg/kg s.c.) or (c) ovine prolactin (4 mg/kg s.c.) on mean $\pm$ s.e.m. (5 rats/group) serum levels of LH and prolactin before and after a single injection of synthetic LH-RH (100 ng via cardiac puncture shown by arrows) at 16:00 h. Blood samples were taken at 12:00, 15:50 and 16:30 h. Vehicle-treated controls are also shown (a). * $P < 0.05$ compared to equivalent 12:00 h value (paired t test).

Experiment 2: effect of sub-chronic hyperprolactinaemia on serum levels of LH and prolactin, pituitary responsiveness to LH-RH in vitro and MBH LH-RH content

Sub-chronic treatment with domperidone depressed serum LH levels compared to those of vehicle-treated controls for a period of 8 days (Text-fig. 3). A pronounced inhibition of LH was observed on the first day of treatment and levels remained uniformly low until Day 6, after which a gradual rise in serum LH was apparent. By Day 9 there was no significant difference between the control and treated groups. Marked hyperprolactinaemia was evident in all the post-treatment samples taken from the domperidone group throughout the period of the experiment. Treatment with domperidone twice daily for 3, 5 or 7 days did not significantly modify in-vitro pituitary responsiveness to LH-RH (Text-fig. 4a), although the responsiveness of the 7-day group did appear to be lower than that of the vehicle-treated controls. A significant inhibition of pituitary responsiveness of pulses of LH-RH was observed, however, after 9 days of domperidone treatment,

Text-fig. 3. Effect of domperidone (4 mg/kg s.c.) administered twice daily at 09:00 and 17:00 h for 9 days on mean $\pm$ s.e.m. serum levels of LH and prolactin in chronically ovariectomized rats (6/group). Blood samples were taken at 09:00 h on Day 1 and daily at 14:30 h. $\dagger P < 0.05$ compared to previous value (paired *t* test). $*P < 0.05$ compared to equivalent control value (unpaired *t* test).

but treatment for a slightly longer period (12 days) did not produce a similar effect (Text-fig. 4b). No significant differences between control and treated groups in MBH LH-RH content were observed after domperidone treatment (Table 1).

Table 1. Effect of domperidone administered twice daily to chronically ovariectomized rats on the LH-RH content (pg/mg wet wt) of the medial-basal hypothalamus

Treatment	Duration of treatment				
	1 day	5 days	7 days	9 days	12 days
Domperidone	234 $\pm$ 48 (4)	212 $\pm$ 30 (4)	224 $\pm$ 32 (6)	169 $\pm$ 37 (4)	245 $\pm$ 38 (4)
Saline	252 $\pm$ 37 (4)	184 $\pm$ 35 (4)	236 $\pm$ 39 (6)	198 $\pm$ 24 (4)	230 $\pm$ 36 (4)

Values are mean $\pm$ s.e.m. for the no. of observations in parentheses.

Discussion

The present study has demonstrated that the inhibition of LH secretion by prolactin in chronically ovariectomized rats occurs within 2–4 h after induction of hyperprolactinaemia by a single treatment with domperidone or ovine prolactin. Significantly reduced circulating LH levels were maintained at least until 16 h after treatment, although levels were returning to control values after 8 h. These results confirm and extend the findings of Flint & Ensor (1981) who obtained significant lowering of circulating LH at 8 h after treatment with ovine prolactin, and explain the absence of an

Text-fig. 4. Effect of domperidone administered twice daily to chronically ovariectomized rats (4 mg/kg s.c. at 09:00 and 17:00 h, and at 09:00 h only on the final day of treatment) on the release of LH from isolated anterior pituitary glands (taken at 11:00 h on the final day) after different durations of treatment. The response to two pulses of LH-RH (10 ng/ml, horizontal bars) was tested. Values are mean + s.e.m. for 4 rats/group. * $P < 0.05$ compared to equivalent control response (Newman-Keuls test).

inhibitory effect when blood samples were taken at 2, 18 or 24 h after domperidone treatment (Carter & Whitehead, 1981c).

The similarity of response attained with domperidone and ovine prolactin treatment indicates that the inhibitory effects observed with domperidone result from enhanced prolactin secretion, rather than a direct anti-gonadotrophic action of domperidone. A direct relationship between serum levels of LH and prolactin after domperidone treatment is also indicated by an approximately inverse parallelism in the levels of the two hormones over the 24-h period of the experiment. The effect of domperidone demonstrated here is in contrast to results obtained with intact female rats (Sarkar & Fink, 1981), in which an acute facilitatory action on LH secretion was observed, an

action attributed to blockade of inhibitory dopamine receptors in the median eminence. The present results therefore indicate that blockade of such receptors in chronically ovariectomized rats does not significantly modify tonic LH secretion.

Suppression of pituitary responsiveness to LH-RH does not appear to explain inhibition of LH secretion, because pituitary responsiveness *in vivo* was unchanged 4 h after treatment with domperidone or ovine prolactin. This finding is at variance with the demonstration of an acute (4–5 h) inhibition of pituitary responsiveness by exogenous ovine prolactin in lactating rats (Muralidhar, Maneckjee & Moudgal 1977). Additional studies are required to confirm that pituitary responsiveness does not play a role in the inhibition of LH secretion demonstrated here, since it is possible that a transient reduction in responsiveness before 4 h may occur. Alternatively, the single blood sample at 30 min after a relatively large LH-RH stimulus may have masked any impairment of pituitary responsiveness.

Sub-chronic hyperprolactinaemia, induced with twice daily domperidone treatment, resulted in a pronounced inhibition of circulating LH levels for a period of 6 days, after which the levels increased, becoming insignificantly different from control levels on Day 9. A similar pattern of inhibition occurred when hyperprolactinaemia was induced with pituitary transplants (Beck & Wuttke, 1977). Although acute hyperprolactinaemia did not impair pituitary responsiveness to LH-RH, a decrease in pituitary responsiveness after sub-chronic treatment with domperidone could cause the inhibition of LH in chronically ovariectomized rats, with a re-establishment of normal responsiveness resulting in the normalization of LH secretion after 7 days. In complete contradiction to this postulate, pituitary responsiveness was not significantly lower than control levels after 3, 5 or 7 days of treatment (although a reduced response was apparent at 7 days), whereas a significantly reduced response was observed after 9 days and a non-significant reduction was again apparent after 12 days of treatment. Paradoxically, therefore, it appears that the 'escape' from inhibition of LH secretion after 7 days is coincident with a fall in pituitary responsiveness to LH-RH. A similar time course study of pituitary responsiveness during hyperprolactinaemia has not been reported, but in another study, in which hyperprolactinaemia was induced by pituitary transplants for 6 days in ovariectomized rats, the pituitary responses were insignificantly different from those of control animals (Vasquez *et al.*, 1980).

Since the changes in pituitary responsiveness during sub-chronic hyperprolactinaemia do not correlate with the inhibition of LH secretion, it appears that an impairment of LH-RH secretion is responsible for the low LH levels. However, the results of the present study failed to produce evidence for a hypothalamic action of prolactin, in that the LH-RH content of the medio-basal hypothalamus was not significantly modified by domperidone treatment. However, experiments by McNeilly, Sharpe, Davidson & Fraser (1978), which also indicated that the effects of hyperprolactinaemia on LH release in male rats was mediated by changes in LH-RH secretion, similarly failed to show any alteration in the hypothalamic LH-RH content. Marchetti & Labrie (1982) showed that hyperprolactinaemia, induced by pituitary transplants, slightly reduced pituitary binding of an LH-RH analogue in ovariectomized rats after 14 days, although concomitant oestrogen treatment markedly enhanced this inhibitory effect and led to much higher plasma prolactin levels. Such results also indicate that hyperprolactinaemia may alter LH-RH secretion, since this peptide is known to regulate its own receptors (Clayton & Catt, 1981). Clearly the relatively crude measurement of hypothalamic LH-RH concentration is not adequate to assess an impairment of LH-RH secretion in hyperprolactinaemia, and further studies involving either direct assessment of LH-RH secretion in hypophysial portal blood or measurement of LH-RH content in highly localized areas of the hypothalamus are required.

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