THE ROLE OF THE HYPOTHALAMUS
IN THE TONIC SECRETION OF PROLACTIN
INDUCED BY OESTROGENS IN THE RAT

By
H. Kato¹,², M. E. Velasco³ and I. Rothchild¹

ABSTRACT

To investigate where oestrogens act to induce an increase in the tonic release of prolactin, the effect of ovariectomy alone or of ovariectomy plus oestradiol benzoate treatment (10 µg/day for 7 days) (OeB10) on the morning prolactin serum level in adult female rats was determined after each of the following procedures: frontal hypothalamic deafferentation (FHD) (a semi-lunar cut at the caudal border of the optic chiasma, separating the anterior hypothalamus from the preoptic area); medial hypothalamic deafferentation (MHD) (a similar cut, but separating the anterior from the posterior half of the hypothalamus); sham deafferentation; an electrolytic lesion of the arcuate-median eminence area (Arcuate-ME lesion); hypophysectomy and pituitary autotransplantation beneath the kidney capsule. The rats were ovariectomized 1 week after the latter procedure, or 3 weeks after the others, and OeB10 or oil vehicle treatment was begun one week after ovariectomy.

FHD, the Arcuate-ME lesion and pituitary autotransplantation markedly increased the basal prolactin level; MHD induced a much smaller...
increase. The level fell after ovariectomy only in the FHD group. A sharp and equal increase in the prolactin level occurred in the FHD, MHD and sham operation groups in response to OeB10; a much smaller and more variable increase occurred in the Arcuate-ME and pituitary autotransplantation groups.

The results suggest that oestrogens increase the tonic release of prolactin only in part by a direct action on the pituitary, and more importantly by acting on the posterior half of the hypothalamus or on areas with lateral, superior, or posterior afferent connections to this site.

The secretion rate of prolactin is markedly increased by oestrogen administration (Neill 1972; Caligaris et al. 1974; Subramanian & Gala 1976; Goodman & Lawson 1977). A single injection of a long-acting oestrogen produces an increase in the basal morning levels of prolactin which lasts for several weeks; on top of these high basal levels, a daily afternoon surge occurs, comparable to that seen in pro-oestrous rats (Subramanian & Gala 1976; Goodman & Lawson 1977). Two different modalities of prolactin hypersecretion can thus be considered: one is an increase in the basal level of prolactin secretion (tonic release), the other a periodic surge of secretion lasting several hours and occurring during the second half of the light period (phasic release). The tonic hypersecretion occurs in injected male and female rats and develops slowly, unless very large doses of oestradiol are administered (Neill 1972; Caligaris et al. 1974). The phasic release is sensitive to much smaller amounts of oestrogen, does not occur in male rats, and is highly dependent on the time of the day (Neill 1972; Caligaris et al. 1974). It seems to be quite certain that the phasic release in response to oestrogens is neurally mediated. Frontal hypothalamic deafferentation (FHD), a semi-lunar cut at the caudal border of the optic chiasma, separating the medial preoptic area from the medial basal hypothalamus, either blocks or considerably reduces the phasic prolactin surges induced by oestrogen in the rat (Neill 1972; Caligaris & Taleisnik 1976). Pharmacologic blockade of either the adrenergic or serotonergic transmitter systems also prevents this response (Subramanian & Gala 1976; Caligaris & Taleisnik 1974).

The site and mechanisms of action for the oestrogen-induced tonic prolactin hypersecretion have not been defined. It does not seem to require intact rostral afferents to the hypothalamus as in the case of the phasic response. Animals with either FHD or suprachiasmatic lesions characteristically have persistent high levels of endogenous oestrogen, and also have elevated serum prolactin concentrations (Blake et al. 1972; Weiner et al. 1972; Bishop et al. 1972a). These levels decline after ovariectomy. The results suggest that oestrogens may act on the hypothalamus caudal to the cut or directly on the pituitary. The administration of oestrogen to these animals, however, led to contradictory results. Some investigators reported blockage of the prolactin
response to oestrogen by FHD (Neill 1972), others found a depressed reaction (Caligaris & Taleisnik 1976) and a third group described a normal response after suprachiasmatic lesions (Bishop et al. 1972b). All of these studies deal with the effect of short-term oestrogen treatments and the first two reports with the afternoon phasic release. The third one does not state the time of the day at which prolactin levels were determined.

The following study was designed to analyze the changes in the morning prolactin baseline (tonic release) after ovariectomy and in response to daily oestrogen injections. The results were compared in rats subjected to the following experimental conditions:

1) Elimination of rostral afferents to the hypothalamus by FHD or medial hypothalamic deafferentations [MHD: a cut caudal to the former, separating the rostral half of the hypophysiotrophic area (HTA) from the median eminence (ME)].

2) Elimination of hypothalamic influences by either hypophysectomy and remote pituitary autotransplantation or by large median eminence-arcuate electrolytic lesions (Arcuate-ME lesion).

**Materials and Methods**

Female Holtzman (Sprague-Dawley) rats of 260–310 g body weight were housed at 25°C under a 14:10 h light:dark schedule (lights on at 05.00 h) with free access to Purina lab chow and water. Vaginal smears were obtained daily from each rat before 10.00 h and only those animals showing at least two consecutive 4-day cycles were employed.

Ether was used for anaesthesia and all operations were done with a clean but not aseptic technique. FHD, MHD and the Arcuate-ME lesions were done as described previously (Velasco et al. 1974). FHD and MHD were performed with a Halasz knife (Halasz & Pupp 1965); the horizontal blade was 1.5 mm and the vertical one was 2.0 mm long. With the animal's head held in a stereotaxic apparatus, the knife was introduced in the mid-sagittal plane and turned 45° to each side once the base of the skull was reached.

Sham operations consisted of lowering the knife to the base of the skull in the same coordinates used for FHD, and withdrawing it without rotation of the knife within the brain. Arcuate-ME lesions were performed with cathodic DC of 2.0 mA for 10 seconds, passed through a nichrome wire electrode insulated except at its flattened tip. Hypophysectomy was done by the parapharyngeal approach on the first day of dioestrus. The aspirated pituitary was then recovered from a trap bottle on the suction line and immediately transferred beneath the capsule of the left kidney.

After FHD, MHD or Arcuate-ME lesions or the sham operation, each rat was caged individually and vaginal smears were recorded daily for the duration of the experiment. Ovariectomy was done 3 weeks after these operations, and 1 week later the rats were divided into two groups; in one of these, each rat received 1 daily sc injections of 10 μg of oestradiol benzoate (OeB10) in 0.2 ml of sesame oil, and in
the other, only sesame oil. The rats subjected to pituitary autotransplantation were handled in exactly the same way except that ovariectomy was done 1 week after operation.

Blood samples for prolactin determination were obtained by cardiac puncture of unanaesthetized rats. Prolactin levels in blood serum samples obtained in this way from cyclic rats were found to be statistically identical to those obtained by decapitation, provided that no more than 30 seconds elapsed between picking up the rat and filling the syringe with the required volume (Kato & Rothchild, unpublished findings). In this study prolactin levels were included in the final data only if the sample was obtained within 20 seconds of picking up the rat. All blood samples were taken between 11.00 and 12.00 h. They were allowed to clot, centrifuged at 4°C and stored at −20°C until assay. Prolactin was determined in duplicate by a double-antibody radioimmunoassay according to the method of Neill & Reichert (1971), and the results expressed in terms of purified rat prolactin standard LER-1382-82 (45.7 IU/mg).

For histological assessment of the site of cut or lesion, the brains were fixed in 10% formalin and studied microscopically in unstained frozen sections. Cuts were made at 40 µm thickness in horizontal planes in the FHD and MHD groups and in para-sagittal planes in the Arcuate-ME lesioned group. Only those rats are included

---

**Fig. 1.**

Serum prolactin levels (mean ± sem) in intact female rats 3 weeks after brain surgery or 1 week after hypophysectomy and pituitary autotransplantation. The prolactin values of normal cyclic female rats in dioestrus are taken as reference. The number near or at the bottom of each column represents the number of rats. *P*-values (Student’s *t*-test) for each experimental group compared to normal dioestrous controls were in each case < 0.001. Abbreviations: CTR (dioestrous controls), FHD (frontal hypothalamic deafferentation), MHD (medial hypothalamic deafferentation), AP-tr (hypophysectomy and pituitary-autotransplantation), ARC-L (arcuate-median eminence lesion).
in the final results in which the location of the lesion or cut could be positively identified, and in which these locations fitted the definitions of FHD, MHD and the Arcuate-ME lesion.

Statistical comparisons of serum prolactin concentrations were made using Student's t-test. A P-value of less than 0.05 was considered significant. The mean rate of increase in serum prolactin was determined by the slope of the prolactin concentration curve from day 8 to 15. The data were compared by analysis of variance.

![Graph showing serum prolactin levels across different groups](image_url)

**Fig. 2.**

The effect of ovariectomy or of oestrogen treatment on the serum prolactin levels of rats with frontal hypothalamic deafferentation (FHD), medial hypothalamic deafferentation (MHD), hypophysectomy and pituitary autotransplantation under the kidney capsule (AP-tr), arcuate-median eminence lesions (ARC-L), and sham FHD (Sham). The prolactin standard had a potency of 45.7 IU/mg. Days of the experiment are represented on the abscissa. Day 1 was the day of ovariectomy; this was done 3 weeks after brain surgery or 1 week after hypophysectomy and pituitary autotransplantation. From days 8 through 14, 10 μg of oestradiol benzoate (OeB10) in sesame oil, or the oil, was injected daily. Each point represents the mean ± SEM of the number of values indicated near each point. The rate of rise of prolactin from day 8 through 15 is expressed as the mean slope ± SEM at the top of each panel. **Statistical analysis.** The mean slopes of AP-tr and ARC-L were significantly different (analysis of variance) from those of FHD, MHD, Sham groups at *P* < 0.05, respectively. *P*-values (Student's *t*-test) compared to pre-treatment value on day 8: *P* < 0.05, **P** < 0.01.
RESULTS

Basal levels of prolactin in the FHD, Arcuate-ME lesion and pituitary autotransplantation groups at the moment of ovariectomy were markedly increased above those of intact cyclic rats; a slight but significant increase was also seen in the MHD group (Fig. 1).

Ovariectomy itself caused a significant reduction in the serum prolactin level only in the FHD group, although the level reached still remained slightly though significantly above that of the MHD and sham operation groups ($P < 0.01$ and $P < 0.001$, respectively) (Fig. 2). Daily OeB10 treatment increased the prolactin levels. The values were significantly higher than those of the oil-treated rats in all groups except the Arcuate-ME one. The rate of increase, determined by the mean slope of the prolactin concentration curve, was significantly smaller in the Arcuate-ME lesion ($P < 0.05$) and in the pituitary autotransplantation groups ($P < 0.005$) than in any of the others. Oil-treatment had no effect on the prolactin level (Fig. 2).

DISCUSSION

Our findings in the FHD rats confirm the view that prolactin hypersecretion is secondary to the continuous secretion of oestrogen induced by FHD (Blake et al. 1972; Weiner et al. 1972; Bishop et al. 1972a). Bishop et al. (1972b) also found, somewhat as we did in the FHD rats, that, two months after ovariectomy, rats with suprachiasmatic lesions had slightly higher prolactin levels than did ovariectomized rats without such lesions. The dramatic rise in the serum prolactin level after the Arcuate-ME lesion or after pituitary transplantation is well-recognized (Bishop et al. 1972a; Chen et al. 1970) and our findings that the former resulted in a greater serum prolactin level than did the latter confirm those of Chen et al. (1970). It is known that pituitary atrophy is considerably greater following transplantation than after hypothalamic lesions. The elevated prolactin levels in both conditions are undoubtedly the consequence of the removal of the pituitary from the inhibitory influence of the hypothalamus and the lack of effect of ovariectomy (Fig. 2), therefore, is not surprising.

The site of production of the prolactin inhibiting factor (PIF) may be co-extensive with the hypophysiotrophic area (HTA) described by Halasz et al. (1965), since intracerebral pituitary grafts have a morphology similar to those grafted under the kidney capsule, except when they were placed in the zone of the hypothalamus corresponding to the HTA (Halasz et al. 1965). MHD separates the rostral half of the HTA from the median eminence. From the results of MHD on indirect criteria of prolactin secretion it was
suggested that the rostral HTA was the main site of production of PIF (Velasco et al. 1974). The small increase in the basal levels of prolactin following MHD (Fig. 1) may indeed be interpreted as secondary to the separation of the pituitary from part of the PIF producing elements; a similar increase occurs after electrolytic lesions of the anterior hypothalamus (Chen et al. 1970). This increase, in contrast to that seen after FHD, does not depend on oestrogens, since it remains the same after ovariectomy (Fig. 2). The much smaller increase than the one which follows Arcuate-ME lesions (Fig. 1), however, indicates that a significant part of the PIF neuronal pool must lie caudal to the site of the MHD cut.

Since the prolactin levels increased in response to oestrogen treatment at almost the same rate in the FHD, MHD and sham operation groups, the action of the oestrogen must obviously not depend on the rostral neural afferents to the hypothalamus, as is the case for the phasic release of prolactin in response to oestrogens (Neill 1972; Caligaris & Taleisnik 1976).

In the rats whose pituitaries were separated from the hypothalamus (i.e., the Arcuate-ME and the pituitary autotransplantation groups) the oestrogen could have had only a direct action on the pituitary in inducing a change in prolactin secretion. Since the rate of increase in the prolactin level in these rats was significantly smaller than it was in the rats in which the pituitary connection with the hypothalamus remained intact, it follows that a very important part of the action of the oestrogen must have been exerted on the hypothalamus. Because of the lack of difference between the sham operation, FHD and MHD rats in the rate of rise of the prolactin level, it also follows that the site of action of the oestrogen must have been either on the portion of the hypothalamus lying between the site of the MHD cut and the median eminence, or on afferents to this area from areas lateral, above or posterior to it.

ACKNOWLEDGMENTS

We wish to thank Helen Wilk and Edward Butler for their help with animal care and Rosa Garnett for preparation of the manuscript. We are grateful to Dr. Richard McCormack of the Schering Corporation for the supplies of oestradiol benzoate, and to Drs. Jimmy Neill and L. E. Reichert, Jr., Emory University, for the antiserum to rat prolactin JDN-10, and for the prolactin for iodination LER-1421-94-97, and rat prolactin standard LER-1382-82, respectively. We also thank Dr. Ann Merrell of the Biometry Department for statistical treatment of the data.

REFERENCES


Received on May 25th, 1978.