Effect of Hypothalamic Extract on Serum Prolactin Levels 24 hr Following Forebrain Removal[1] (37655)

CAROL TURPEN,[2] A. ARIMURA, AND J. DUNN
(Introduced by M. Hess)

Department of Anatomy, Louisiana State University Medical Center, and Department of Medicine, Tulane University School of Medicine, Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, New Orleans, Louisiana 70112

Most experimental evidence indicates that prolactin release is tonically inhibited by the hypothalamus (1). Ablation of the median eminence (2), transection of the pituitary stalk (3, 4), transplantation of the anterior pituitary (2) and use of certain CNS-depressant drugs (5, 6) result in increased blood levels of prolactin. Additionally, both *in vitro* and *in vivo* studies (6) have shown decreased prolactin release following administration of hypothalamic extracts.

Although it has been assumed that the inhibitory factor contained in hypothalamic extracts exerts its effect directly on the pituitary lactotropes, recent data suggests that the inhibitory influence of the crude hypothalamic extracts is dependent upon the presence of endogenous hypothalamic prolactin inhibitory elements (7). Elevated serum prolactin levels in rats subjected to perphenazine–pentobarbital pretreatment or extensive ablation of the hypothalamus were not decreased following infusion of hypothalamic extracts (7). Thus, it may be that the inhibitory effect of hypothalamic extracts on prolactin release in intact animals (7, 8) is via an indirect influence on endogenous hypothalamic inhibitory elements; *i.e.*, by facilitating the release of endogenous prolactin release-inhibitory hormone (PRIH).

The present experiments were designed to further assess the inhibitory nature of hypothalamic extracts on serum prolactin levels in rats subjected to forebrain removal. Brain removal was employed to insure the absence of endogenous hypothalamic inhibitory factors.

[1] Supported by NIH Grants NS08929 and AM-09094.

[2] N.S.F. Predoctoral Fellow No. GZ-2011.

Materials and Methods. Animals used in these studies were adult male (240–300 g) rats (Southern Farms) acclimated to controlled temperature (23 ± 1°) and lighting (fluorescent illumination from 0400 to 1800) for at least 2 wk prior to treatment. Purina laboratory chow and tap water were available *ad libitum*.

Rats with pituitary islands (PI) were prepared by rapidly removing with suction all forebrain structures (including median eminence and pituitary stalk) rostral to the midbrain, leaving an isolated pituitary. Figure 1 illustrates the amount of brain removed in these preparations. Intact animals served as controls. Because PI rats stopped eating and drinking after forebrain removal, food and water were withheld from control animals. Following surgery all rats received intraperitoneally 10 ml of 5% dextrose in physiological saline.

Stalk–median eminence extracts (SME) were prepared from pooled frozen hypothalamic fragments consisting primarily of median eminence and pituitary stalk. Subsequent to thawing, SME were homogenized in 0.1 *N* HCl (50 μl/SME), and centrifuged at 15,000 rpm for 20 min at 4°. The supernatant was diluted with physiological saline to a concentration of 5 SME equiv/0.55 ml.

Serum prolactin levels were measured by radioimmunoassay using the NIAMD-rat-prolactin RIA kit, according to the method of Niswender *et al.* (10) modified by the use of ^{125}I for iodination.

Effect of forebrain removal on serum prolactin levels. To determine the effect of complete forebrain removal on serum prolactin levels, "nonstress" blood samples were obtained from control and PI rats by rapid de-

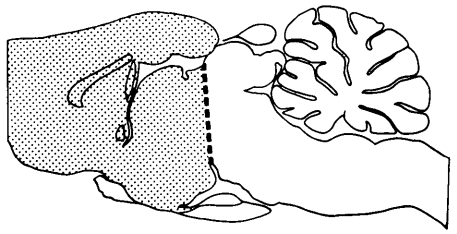

FIG. 1. Diagram of PI preparation; stippled area indicates brain removed.

capitation (<20 sec following initial handling) 24 hr after forebrain removal. Trunk blood was collected and allowed to clot. The serum was removed, frozen and stored at −20° until assayed. To further standarize conditions and minimize the overt stress associated with blood collecting procedure, animals were removed individually to an adjacent preparation room for decapitation. All experiments were performed between 0800 and 0900 hr.

Effect of SME on serum prolactin levels in rats with forebrain removed. The second study was undertaken to assess the effect of SME on pituitary prolactin release. Twenty-four hours following surgery, PI and intact rats were anesthetized with ether, the jugular vein was exposed and a preinfusion blood sample was obtained. Immediately thereafter, a cannula was placed in the jugular vein and SME or acid–saline vehicle was infused for 30 min (0.55 ml/animal). Forty minutes following initiation of SME infusion, animals were decapitated and trunk blood was collected for serum prolactin determination.

Subsequent to decapitation the heads were placed in 10% formalin. Following fixation, they were decalcified in 10% formic acid and processed for histologic examination of *in situ* brain and pituitary.

All treatments were assigned and performed according to a randomized block design. Statistical probabilities were determined using Student's *t* test.

Results. Effect of forebrain removal on serum prolactin levels. Twenty-four hours following surgery, serum prolactin levels in PI rats were significantly ($p < 0.01$) higher than "nonstress" levels in intact controls (Fig. 2).

Effect of SME on serum prolactin levels in rats with forebrain removed. Figure 3 illustrates the effect of SME infusion on serum prolactin levels. As indicated, infusion of SME in intact animals produced the expected decrease ($p < 0.02$) in circulating prolactin levels. In contrast, SME did not have a significant effect on serum prolactin levels in PI rats. Infusion of saline did not have an effect on serum prolactin levels in either intact or PI animals.

Histology. The completeness of forebrain removal in all PI preparations was verified histologically. Small infarcts were observed in a few island preparations. Necrotic tissue, when present, was located centrally in either one or both of the lobes of the anterior pituitary.

Discussion. The finding that serum prolactin levels were increased following forebrain removal is consistent with the results of previous studies which have shown that interruption of hypothalamic–pituitary contiguity results in increased prolactin secretion (1–4, 7). Ablation of the hypothalamus (7) or median eminence (2), pituitary stalk section (3, 4) or transplantation of the pituitary (2) produces markedly increased circulating levels of prolactin. In the present study, serum prolactin levels in PI rats showed a twofold increase above the nonstress levels of intact rats.

Although it is generally accepted that hypothalamic extracts have a direct inhibitory effect on the pituitary lactotropes, several recent studies suggest that the crude extracts

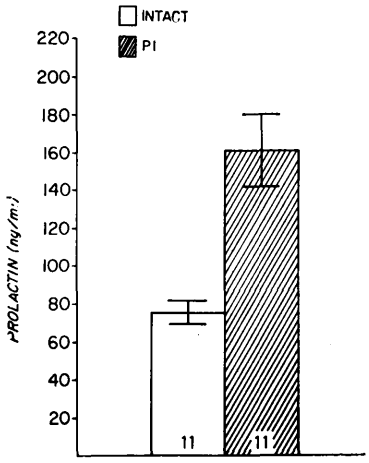

FIG. 2. Serum prolactin levels 24 hr after forebrain removal. In this and the subsequent figure, number of animals per treatment group is indicated at the base of the column; vertical lines indicate standard error.

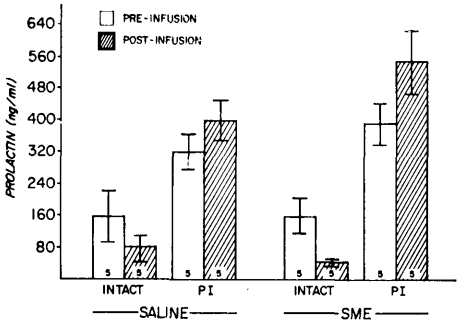

FIG. 3. Serum prolactin levels in intact and PI preparations before and after intravenous infusion of SME or saline.

may not act at the pituitary level to inhibit prolactin secretion (7, 11, 12). Serum prolactin levels in hypophysectomized rats with pituitary autografts to the renal capsule were not suppressed with hypothalamic extracts (11, 12). Nor were the elevated serum prolactin levels in rats subjected to perphenazine–pentobarbital pretreatment or extensive ablation of the hypothalamus decreased 40 min after SME infusion (7). However, intact animals given SME showed marked prolactin suppression (7). Consistent with the latter observations, ether-anesthetized intact rats in the present study showed a significant reduction in prolactin levels after SME infusion, but the high prolactin levels observed 24 hr after forebrain removal were not significantly influenced by SME. The possibility that pituitary sensitivity to the extract was altered by forebrain removal cannot be excluded. However, the finding that PI preparations show increased LH and corticosterone levels following infusion of SME (unpublished data) suggests that altered pituitary sensitivity does not account for the lack of suppression in PI rats.

Collectively, these observations offer considerable evidence that the *in vivo* inhibitory influence of hypothalamic extracts on prolactin release is dependent on the availability of endogenous PRIH elements. Decreased blood levels of prolactin following SME infusion in intact animals may reflect SME-evoked PRIH release similar to that described for vasopressin-evoked ACTH release (13).

Summary. The effect of intravenous infusion of stalk–median extracts (SME) on serum prolactin levels was studied in male rats 24 hr after complete forebrain removal. Brain removal was used to produce pituitary islands (PI) and insure the absence of endogenous hypothalamic inhibitory factors. Twenty-four hours after forebrain removal, prolactin levels showed a twofold increase above "nonstress" levels in intact animals. In ether-anesthetized intact male rats, infusion of the hypothalamic extract (5 SME equivalents) markedly decreased prolactin levels. In contrast, prolactin levels were not significantly reduced in PI preparations 40 min following initiation of infusion. The results suggest that the decreased blood levels of prolactin observed after administration of hypothalamic extract to intact animals may reflect the release of endogenous prolactin release inhibitory hormone (PRIH), evoked by the extract, and not a direct inhibitory effect on pituitary prolactin secretion.

We express our appreciation to Laurel Patterson and Jacqueline Skaggs for their excellent technical and secretarial assistance.

1. Meites, J., and Nicoll, C. S., Annu. Rev. Physiol. **28**, 59 (1966).

2. Chen, C. L., Amenomori, Y., Lu, K. H., Voogt, J. L., and Meites, J., Neuroendocrinology **6**, 220 (1970).

3. Nikitowitch-Weiner, M. B., Endocrinology **77**, 658 (1965).

4. Turkington, R. W., Underwood, J. J., and Van Wyk, J. J., N. Engl. J. Med. **285**, 707 (1971).

5. Lu, K. H., Amenomori, Y., Chen, C. L., and Meites, J., Endocrinology **87**, 667 (1970).

6. Blackwell, R., and Guillemin, R., Annu. Rev. Physiol. **35**, 357 (1973).

7. Arimura, A., Dunn, J. D., and Schally, A. V., Endocrinology **90**, 378 (1972).

8. Amenomori, Y., and Meites, J., Proc. Soc. Exp. Biol. Med. **134**, 492 (1970).

9. Kamberi, I. A., Mical, R. F., and Porter, J. C., Endocrinology **88**, 1294 (1971).

10. Niswender, G. D., Chen, C. L., Midgley, A. R., Meites, J., and Ellis, S., Proc. Soc. Exp. Biol. Med. **130**, 793 (1969).

11. Arimura, A., Proc. 52nd Meet. Endocrine Soc., Abstr. 182 (1970).

12. Acheson, R. D., and Leavitt, W. W., Biol. Reprod. **6**, 258 (1972).

13. Hedge, G. A., Yates, M. B., Marcus, R., and Yates, F. E., Endocrinology **79**, 328 (1966).

Received July 5, 1973. P.S.E.B.M., 1973, Vol. 144.