PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 104–108 (1974)

The Ability of Hypothalamic Extracts to Lower Blood Prolactin Levels in Lactating Rats (38052)

E. KUHN,[1] L. KRULICH, C. P. FAWCETT, AND S. M. MCCANN[2]

Department of Physiology, University of Texas Southwestern Medical School, Dallas, Texas 75235

Crude or partially purified hypothalamic extracts can inhibit the release of prolactin by pituitaries incubated *in vitro* (1–3). *In vivo* they have been reported to block the suckling-induced decline in pituitary prolactin concentration (4–6). This activity has been purified (6) and attributed to a prolactin-inhibiting factor (PIF) in hypothalamic extracts.

Few observations have been made of the effect of these extracts on blood prolactin levels. Watson *et al.* (7) reported that crude rat hypothalamic extracts could lower plasma prolactin concentration in normal male rats within eight minutes of their injection, and Amenomori and Meites (8) have reported lowering of prolactin in proestrous rats one and four hours after injection of hypothalamic extracts. On the other hand, Arimura *et al.* (9) found that cerebral cortical as well as hypothalamic extract lowered prolaction in pentobarbital-anesthetized rats, and Valverde *et al.* (10) found no lowering, but instead a rise in male rats treated with estrogen and injected with porcine hypothalamic extract. Consequently, it appeared of interest to determine if hypothalamic extracts could alter the suckling-induced rise in blood prolactin levels in lactating females. Suckling has been shown to be a powerful stimulus to prolactin release (11).

Materials and Methods. Animals. Lactating female Holtzman rats were obtained seven to nine days after delivery and the number of pups was adjusted to eight per litter upon arrival. Animals were housed individually in an air-conditioned, light-controlled room (lights on 5 a.m.–7 p.m.) and given free access to laboratory chow and water.

Preparation of hypothalamic extracts. Batches (150–500) of hypothalami from adult male Sprague-Dawley rats (200–250 g) were collected onto dry ice and lyophilized. The dry weight of tissue averaged 3 mg per hypothalamus. The tissue was homogenized in acetone and the dry powder homogenized in five volumes of a mixture of 50% ethanol and 50% 0.2 *N* acetic acid. After centrifuging (11,500*g*, 30 min), the supernatant was taken to dryness on a rotary evaporator at 30°C. The residue was dissolved in 0.1 *N* acetic acid (10–20 ml per 100 hypothalami, a slight amount of insoluble material was centrifuged and discarded), then reduced in volume and washed by ultra-filtration through a Diaflo UM-2 membrane (Amicon Corporation Inc.) with 0.1 *N* acetic acid under pressure until approximately 100 retentate volumes had been collected. The filtrate was rotary evaporated, then lyophilized. The product was taken up in water and stored frozen at a concentration suitable for use.

Two preparations were used. The first one (extract A) contained 24 hypothalamic

[1] Supported by the National Research Foundation (Belgium) and NIH grant #HD 05151. Present address: Catholic University of Leuven, Zoological Institute, Laboratory of Endocrinology, Naamsestraat 61, B-3000 Leuven, Belgium.

[2] Supported by grants from NIH (#AM 10073 and #HD 05151), the Ford Foundation and the Robert A. Welch Foundation.

equivalents per milliliter; the second (extract B) contained 15 hypothalamic equivalents per milliliter.

Testing of extracts. The prolactin-inhibiting activity of the hypothalamic extracts was evaluated both *in vitro* and *in vivo*. For the *in vitro* experiments, anterior lobes were cut longitudinally into halves, and the halves were distributed amongst 25 ml Erlenmeyer flasks such that each flask contained four pituitary halves. After a preincubation period of thirty minutes in medium 199, the medium was replaced with fresh medium containing neutralized hypothalamic extract and the incubation was continued for three hours. Incubations were carried out in a Dubnoff metabolic shaker under an atmosphere of 95% O_2–5% CO_2, with shaking at 60 cycles per min. At the termination of the incubation, the incubation media were frozen prior to dilution and assay of prolactin.

For the *in vivo* experiments, lactating rats were used nine to 16 days post-partum. They were separated from their litters for eight hours and then returned to them. Injections of extracts were made ip either prior to or after reuniting the dams with the pups. Blood samples for the determination of prolactin were obtained at various times by clipping the tip of the tail with sharp scissors and expressing several drops of blood by gentle pressure onto a piece of parafilm. One hundred μl of blood were then transferred into buffer (phosphate-buffered saline, 1% egg albumin, pH 7.0). The volume of buffer was adjusted to obtain the appropriate dilutions, then centrifuged, and the supernatant was used for prolactin determination.

Prolactin in either blood or incubation media was measured by radioimmunoassay using the NIAMD kit. Results were expressed in terms of the RP-1 rat prolactin standard. Statistical significance of results was calculated by Student's *t test*. Pressor and milk-ejecting activities of the extract were estimated by the methods of Dekanski (12) and Bisset *et al.* (13), respectively.

Results. In vitro experiments. There was a significant decrease of prolactin output by pituitaries incubated *in vitro* with the filtrate which passed through the UM-2 membrane (Table I). The minimal effective dose (MED) in hypothalamic equivalents was 0.4, and a graded response resulted from increasing doses. On the other hand, the material retained behind the filtrate (retentate) had no significant effect even with the highest dose tested.

TABLE I. Effect of UM-2 Filtrate and Retentate of Rat Hypothalamic Extract on Prolactin Release *in Vitro* (Mean of Six Readings on Duplicate Flasks).

	Hypothalamic equivalents/ml	Medium prolactin ng RP-P-1/ml
Control	—	2610
UM-2 Retentate	0.4	2430
UM-2 Retentate	2	3340
UM-2 Retentate	10	2420
UM-2 Filtrate	0.4	2290
UM-2 Filtrate	2	1660
UM-2 Filtrate	10	1380

In vivo experiments. As previously reported (11), suckling induced a dramatic rise in prolactin such that blood prolactin levels had increased more than tenfold within twenty minutes (Table II). The elevated values were maintained during the one hour duration of suckling.

When six hypothalamic equivalents of extract A were administered prior to the start of suckling, there was a significant reduction in blood prolactin as compared to

TABLE II. Effect on Blood Prolactin Levels of a Single Injection of Six Hypothalamic Equivalents (0.25 ml) of Hypothalamic Extract (HE)—A ip before the Start of Suckling.

Time	Treatment	Blood prolactin (ng/ml SE)
Presuckling	Saline	19.5 ± 1.74
sample	HE	17.2 ± 1.74
20 min	Saline	249 ± 15.78
of suckling	HE	142 ± 28.56[a]
40 min	Saline	318 ± 18.78
of suckling	HE	316 ± 25.20
60 min	Saline	283 ± 12.2
of suckling	HE	293 ± 16.9

[a] $P < .01$ versus saline-injected rats (9 rats/group).

TABLE III. Effect of HE-A (0.25 ml), Cortical Extract or 100 mU of Oxytocin on Blood Prolactin Levels in Suckling Rats 30 min After the Respective Injections.

Treatment	Blood prolactin (ng/ml ± SE)
HE	156 ± 41.3[a]
Cortical Extract	293 ± 39.2
100 mU Oxytocin	330 ± 11.2

[a] $P < .01$ versus oxytocin-treated; $P = .05$ versus cortical extract-injected; (5 rats/group).

the saline-injected controls at 20 min after the onset of suckling, but the inhibitory effect had disappeared by 40 min.

In a second experiment, animals were injected prior to the onset of suckling with either the same dose of hypothalamic extract, a similar dose of cortical extract, or 100 mU of oxytocin (Table III). Blood prolactin in both control groups of animals was elevated and similar, but the levels in the animals injected with the hypothalamic extract were significantly less.

To determine whether hypothalamic extract could lower already elevated levels of prolactin in suckling rats, the extract was injected after 30 min of suckling (Table IV). Hypothalamic extract significantly lowered blood prolactin on measurement 15 min later on comparison with the values of controls injected with 100 mU of oxytocin, but the inhibitory effect had disappeared at 45 min after injection.

In another experiment varying doses of the second hypothalamic extract (extract B) were injected after 30 min of suckling (Table V). Only the highest dose, equivalent to ten hypothalami, lowered prolactin significantly at 15 min after injection and again the effect had vanished by 45 min post-injection.

TABLE IV. Effect of 0.25 ml of HE-A or 100 mU Oxytocin Injected After 30 min of Suckling.

Time	Treatment	Blood prolactin (ng/ml ± SE)
30 min of suckling	100 mU oxytocin	307 ± 34.5
	HE	303 ± 24.8
45 min of suckling	100 mU oxytocin	284 ± 13.3
	HE	189 ± 19.8[a]
75 min of suckling	100 mU oxytocin	286 ± 19.5
	HE	311 ± 37.5

[a] $P < .01$ versus oxytocin-injected (8 rats/group).

TABLE V. Effect of Varying Doses of HE-B Injected After 30 min of Suckling on Blood Prolactin Levels in Suckling Rats.

Dose of HE	Time of suckling	Blood prolactin (ng/ml ± SE)
0.6 ml HE	30 min	277 ± 24.73
	45 min	176 ± 39.27[a]
	75 min	335 ± 52.90
0.2 ml HE	30 min	380 ± 38.91
	45 min	354 ± 34.85
	75 min	351 ± 29.60
0.07 ml HE	30 min	332 ± 17.20
	45 min	308 ± 5.44
	75 min	309 ± 15.12

[a] $P < .05$ versus 30 or 75 min (5 rats/group).

An additional experiment was performed with two injections of the high dose of extract B (Table VI). The first dose was injected just prior to replacing the pups and the second was given after 20 min of suckling. The suckling-induced rise in prolactin was partially blocked as in the earlier experiments, and the levels remained low in the 20 min samples after the second injection. By 60 min of suckling, 40 min after the second injection, levels had once again returned to the control value observed with cortical extract. Thus, the second injection of hypothalamic extract was able to maintain the suppression of prolactin longer than a single dose.

In order to provide an additional control for possible oxytocin contamination of the extract, this polypeptide was injected at 30 min of suckling and found to be ineffective in lowering prolactin at either a 50- or 250-mU dose (Table VII).

Examination of the pressor activity of the extracts revealed that they had only a vasodepressor effect. Consequently, it was not possible to quantitate the amount of vasopressin which was present. Measurements of milk-ejecting activity indicated that extract A contained 10.3 mU/ml of oxytocin and extract B contained 2.9 mU/ml.

Discussion. The present results clearly show that ultrafiltrates of rat hypothalamic

TABLE VI. Effects of Repeated Injections of 0.6 ml of HE-B on Prolactin Levels. First Injection Before the Start of Suckling, Second Injection After 20 min of Suckling.

Time	Treatment	No. of rats	Blood prolactin (ng/ml ± SE)
Presuckling	Cortical E	6	13.2 ± 4.02
sample	HE	7	11.5 ± 2.58
20 min	Cortical E	8	232 ± 27.5
of suckling	HE	7	135 ± 27.3[a]
40 min	Cortical E	7	226 ± 20.03
of suckling	HE	8	159 ± 21.81[a]
60 min	Cortical E	6	239 ± 37.5
of suckling	HE	8	238 ± 40.3

[a] $P < .01$ versus cortical extract-injected.

extract contain prolactin-inhibiting activity whether this activity is evaluated *in vitro* or *in vivo*. The MED of approximately 0.4 hypothalamic equivalents *in vitro* was much less than the MED of six hypothalami required *in vivo* with the same preparation. The lower sensitivity *in vivo* may be related to the short half-life of prolactin-inhibiting factor (PIF) in the circulation and to the fact that only a small fraction of the injected extract would reach the anterior pituitary gland.

In the *in vivo* experiments, the extract was capable of attenuating the rise in prolactin which followed the onset of suckling and of lowering already elevated levels of prolactin in nursing animals. In this latter instance, prolactin levels were reduced almost by a factor of two, fifteen minutes after the injection of the extract. Since the half-life of prolactin is estimated to be approximately ten minutes (7), this would indicate that the extracts had markedly reduced but not completely shut off prolactin release by the pituitary of the suckled dams.

TABLE VII. Effect of 50 or 250 mU of Oxytocin Injected After 30 min of Suckling on Levels of Blood Prolactin (5 rats/group).

Dose	Time of suckling	Blood prolactin (ng/ml ± SE)
50 mU oxytocin	30 min	301 ± 21.2
	45 min	390 ± 68.8
	75 min	308 ± 19.9
250 mU oxytocin	30 min	294 ± 29.6
	45 min	302 ± 9.4
	75 min	249 ± 25.6

In addition to requiring a much larger dose, the effect of the extract given *in vivo* was transient, lasting no more than 30 min. The effect could be prolonged by injecting a second dose of extract. The short duration of action is probably related to a short half-life of PIF in the circulation. The failure of Amenomori and Meites (8) to observe an effect of hypothalamic extract in nursing rats is almost certainly due to the fact that they measured serum prolactin three hours after injection of the extracts.

In contrast to the recent report by Arimura *et al.* (9), in which it was claimed that cortical extracts were as effective as hypothalamic extracts in lowering prolactin, there was no response to cortical extracts in the present experiments. Furthermore, the doses of oxytocin injected, which were greater than the quantity of oxytocin contained in the extracts, failed to alter blood prolactin. It would appear that the lowering of prolactin seen *in vivo* was due to a PIF in the hypothalamic extracts. The results are in good agreement with the earlier work with male rats reported by Watson *et al.* (7), and also are in agreement with the results of infusion of hypothalamic extracts into the portal vessels of male rats recently reported by Kamberi *et al.* (14). The results are as would have been expected from the early work of Grosvenor *et al.* (4–6), in which it was shown that hypothalamic extracts could block suckling-induced depletion of pituitary prolactin.

Summary. (1) Ultrafiltrates of partially purified rat hypothalamic extract were capable of inhibiting prolactin release *in*

vitro. (2) These same extracts at a higher dose partially prevented the suckling-induced rise in blood prolactin concentration and lowered the already elevated levels present in suckling rats. (3) The *in vivo* response to hypothalamic extracts was of short duration and had disappeared within 45 min of the injection. (4) Under these conditions, similarly prepared cortical extracts or oxytocin in doses higher than those found in the extracts had no effect. (5) It is concluded that partially purified hypothalamic extracts from the rat contain a prolactin-inhibiting factor.

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Received Sept. 4, 1973. P.S.E.B.M., 1974, Vol. 146.