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Gonadotropin-Releasing Hormone in Milk

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Abstract. *The hypothalamic hormone gonadotropin-releasing hormone (GnRH) has been found in milk of man, cow, and rat. Radioimmunoassays of acidified milk indicate concentrations of GnRH ranging between 0.1 and 3 nanograms per milliliter. Multistep extractions, followed by electrophoresis, reveal gonadotropin-releasing activity in the fraction that comigrates with the GnRH-marker. A second hypothalamic hormone, thyrotropin-releasing hormone, is present in milk at a much lower concentration. "Milk-GnRH" may influence the secretion of the gonadotropic hormones in neonates.*

The gonadotropin-releasing hormone (GnRH), which activates gonadal function by promoting gonadotropin secretion from the anterior pituitary, is present in minute amounts in the hypothalamus [approximately 100 ng and 5 ng in human and rat, respectively (*1*)]. Measurement of the peptide in the peripheral circulation has presented many difficulties (*2*) and only elaborate extrica-

Table 1. Scheme of extraction of GnRH from milk.

Step	Brief description	Recovery of 1 μc of [^{3}H]GnRH (%)
1	Lyophylized skim milk (4.5 liters) or 400 g of powdered milk was stirred with 3.5 liters of acetic acid (2*N*) in methanol for 48 hours at room temperature	100
2	Filtration through Whatman No. 1 filter paper, evaporation of solvent under vacuum, and extraction of residue with 200 ml of acetic acid in methanol	99
3	Filtration, evaporation, and extraction in methanol (200 ml)	
4	Filtration of suspension and evaporation. Residue redissolved in 250 ml of acetic acid (1*N*)	
5	Three extractions with ether (discarded); acetic acid phase evaporated	98
6	Residue redissolved in 400 ml of water and ultrafiltrated through Amicon UM 05 membrane*	
7	Residue (40 ml) lyophylized and redissolved in 10 ml of acetic acid in methanol	85
8	Electrophoresis of the material at *p*H 3.5 for 10 minutes at 10 volt/cm and 60 minutes at 60 volt/cm; extraction of area containing radioactive GnRH marker with acetic acid (1*N*) and removal of solvent by evaporation under reduced pressure	10

*Ultrafiltrate was processed like the residue through steps 7 and 8 and checked for TRH.

tions of plasma from the hypothalamo-hypophyseal portal system of the rat have yielded meaningful results: less than 30 pg/ml on diestrus and 115 ± 26 pg/ml on proestrus (*3*). We have sought GnRH in other biological fluids such as cerebrospinal fluid—which was found to be devoid of the hormone in normal human subjects—and in milk.

GnRH was first detected in pasteurized cows' milk previously acidified to *p*H 3. Amounts measured by radioimmunoassay ranged between 0.5 and 3 ng/ml. As serum proteins have been reported to interfere with GnRH radioimmunoassay (*2*), chemical identification of the GnRH-like material was attempted.

Gonadotropin-releasing hormone labeled with tritiated pyroglutamate (1 μc, New England Nuclear; 23.5 c/mmole) was added to powdered milk (Four Cows, Dutch Domo-Bedum, Beilen; 400 g, equivalent to 3 liters) or to pasteurized skim milk (Tnuva; 4.5 liters), and GnRH was extracted as shown in Table 1. The area of the electrophoresis paper encompassing the radioactive GnRH marker (Step 8) was extracted; its GnRH content was determined by radioimmunoassay and its gonadotropin-releasing activity assessed by bioassay in vitro. Radioimmunoassay of this electrophoretic fraction indicated the presence of 670 ng of GnRH. If one considers the 10 percent recovery of the labeled hormone (Table 1) and the amount of starting material (4.5 liters), this value corresponds to 1.5 ng of GnRH per milliter of milk; powdered milk was found to contain a similar amount of GnRH. "Milk-GnRH" possesses the inherent biological property of the native hypothalamic hormone, that is, induction of the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Table 2). Moreover, exposure to antiserum directed against the synthetic peptide (*4*) markedly reduces the biological activity of milk-GnRH, which denotes its immunological similarity to the native hormone. A second hypothalamic peptide, thyrotropin-releasing hormone (TRH), is present in milk at a much lower concentration (approximately 1/20 of that of GnRH), although the hypothalamus contains as much TRH as GnRH (*1*).

Table 2. Effect of milk-GnRH on gonadotropin secretion from rat pituitaries in vitro. Pituitaries from 12-day-old male rats were incubated for 90 minutes in 1 ml of Krebs-Ringer bicarbonate medium (*p*H 7.4) containing 1 mg of glucose per milliliter (*10*) and the agents listed. Hormone release was determined by bioassay (*11*).

Treatment	Hormone release (μg/ml)	
	LH	FSH
Control	0.42 ± 0.04*	0.42 ± 0.07
Synthetic GnRH (1 ng)	2.97 ± 0.15†	1.55 ± 0.06†
Milk-GnRH (1 ng)‡	3.75 ± 0.32†	2.76 ± 0.19†
Milk-GnRH plus antiserum§	1.42 ± 0.25‖	0.83 ± 0.22‖

*Mean ± standard error for 12 to 20 determinations of LH and six of FSH. †Significantly different ($P < .01$) from control (Student's *t*-test). ‡As determined by radioimmunoassay after electrophoretic separation. §Prior preincubation overnight with 20 μl of antiserum to GnRH. ‖Significantly different ($P < .01$) from milk-GnRH (Student's *t*-test).

Milk-GnRH may have a physiological role in inducing the elevated gonadotropin levels found in serums of human and rat neonates (*5*). When suckling of female pups is prevented, by separating them from their mothers for 3 hours, the concentration of LH in their serum is significantly lower than that of their suckling littermates (34.4 ± 2.9 as opposed to 125.0 ± 24.7 ng/ml; $P < .001$). This suggests that some of the milk-GnRH is absorbed from the gut of the immature rat in a biologically active form. The assumption that milk is the source of this gonadotropin-releasing activity is strengthened by the fact that in the rat, at least, the neonate pituitary is not yet functionally linked to the hypothalamic source of GnRH (*6*). Furthermore, the hypothalamus itself in this species undergoes sex-differentiation only during the first week of life, and milk-GnRH may be involved in the masculinization of the male hypothalamus by causing the release of testicular steroids which act on the brain (*7*). In the female rat, the ovary does not respond to gonadotropins during the first week of life (*8*).

The presence of considerable amounts of GnRH in milk raises not only the question of its role there but also of its origin. Somatostatin, a so-called hypothalamic hormone, has been found also in the pancreas and is assumed to be synthesized there (*9*). The high concentration of GnRH in milk, which greatly exceeds that in serum, implies either an active concentrating mechanism in the mammary gland or an additional, extrahypothalamic origin for this peptide.

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11. The release of LH and FSH was determined by radioimmunoassay, with kits provided by the National Institute of Arthritis, Metabolism and Digestive Diseases Rat Pituitary Hormone Program. Results are expressed in terms of the RP-1 reference preparations.
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