PHYSIOLOGY

Impact of Low-Molecular Ovarian Peptides on Reproductive Function Inhibited by Partial Food Deprivation

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A preparation of total low-molecular peptides isolated from bovine ovaries by acetic acid extraction was tested for its impact on the reproductive function of rats inhibited by partial food deprivation. Three five-day courses of once-daily injections of the preparation at 5 mg/kg restored the number of growing follicles and ovulating ova, estrous cycle duration, and uterine and ovarian weight to their levels found in intact rats.

Key Words: *low-molecular ovarian peptides; reproductive function; partial food deprivation*

Recent research has shown that regulatory peptides participate not only in the intratissue regulation of ovarian, pituitary, and hypothalamic functions but also, apparently, in the systemic regulation of reproductive function. Thus, a number of peptides (inhibins, activins, follistatins, regulatory follicular peptide, etc.) exerting diverse effects on central neuroendrocrine structures were isolated from ovarian tissue [5,7,11,13] and a correlation was found to exist between the blood serum concentration of some of these peptides and various pathological conditions of the reproductive system [5,6].

There is new evidence that regulatory (low-molecular) peptides are instrumental in adapting the body to food deprivation [4,12] and that one substance mediating the inhibition of reproductive function during starvation is likely to be insulin-like growth factor I [9-10]. In view of this, the present study was undertaken to evaluate the biological activity of low-molecular peptides (LMP) on an animal model of reproductive function inhibited by starvation.

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MATERIALS AND METHODS

Total LMP (mol. wt. <15 kD) extracted from bovine ovaries by acetic acid were tested for their impact on the reproductive function of rats. The LMP preparation used was a dry white powder packed into vials (10 mg per vial) under sterile conditions and dissolved in isotonic NaCl solution immediately before being injected into rats. The experiment was started after a 2-3 week period of adaptation to laboratory conditions with the establishment of regular estrous cycles as determined by daily vaginal smears taken in the morning. A total of 32 random-bred female rats aged 3-4 months from the Rappolovo Nursery (body weight 180-220 g) were used.

Reproductive function was inhibited in the test rats by partial food deprivation, i.e., by their chronic underfeeding (alternating free access to food for 24 h with no food at all for 24 h), which caused body weight loss and prolonged the estrous cycle. While

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on this feeding regime, the rats received three courses of 5 once-daily injections of LMP in doses of 0.02, 2 and 5 mg/kg, respectively, at 10-day intervals between the courses which were followed by a single injection of the preparation at 5 mg/kg (Table 1). After each course and the final injection, the duration of the estrous cycle was recorded. The experiment was terminated by euthanasia of the rats under light ether anesthesia during estrus to weigh their uteri and ovaries and count the number of ovulated oocytes.

After weighing, the ovaries were fixed in Bouin's fluid and embedded in paraffin to prepare serial 5-μ sections. Every 10th hematoxylin and eosin-stained section was examined, counting normal and atretic cavitary follicles of different size using the oocyte nucleus as the marker. The diameter of a follicle was taken to be the average result of measuring its two mutually perpendicular dimensions. A follicle was regarded as atretic if at least three granulosa cells with obvious signs of karyorrhexis (pyknotic cells) were detected in the largest transverse section through the oocyte.

The results were analyzed by Student's t test, setting statistical significance at $p<0.05$.

RESULTS

The regime of partial food deprivation caused a two-fold increase in cycle duration in the rats injected with physiological saline instead of LMP (Table 1). The 0.2 mg/kg dose of LMP had virtually no effect on cycle duration in the fasted rats, the 2 mg/kg dose only tended to shorten the cycle, while the 5 mg/kg dose shortened it to a level close to that recorded for the intact rats; the single injection of LMP at 5 mg/kg was also effective in this respect (Table 1).

The chronically underfed rats given saline lost some 20% of their body weight, whereas those given LMP lost only about 10% (Table 2). As shown in this table, the body weight of the latter rats was, on average, 28 g greater than in the saline-treated fasted rats — a figure exceeding by at least three orders of magnitude the total dose of LMP received by each rat (~8 mg). The alimentary contribution of the peptides was thus negligibly small.

Partial food deprivation significantly reduced uterine and ovarian weight as well as the number of ovulating oocytes in the saline-treated group, but not in the LMP-treated group, where all three parameters were close to their control values (intact group); LMP had little if any effect on the parameters under study in the intact (normally fed) rats (Table 2).

Numerical data from the histological analysis of rat ovaries at the estrus stage are presented in Table 3. It can be seen that the total number of growing follicles in the saline-treated fasted rats was significantly smaller (by almost 50%) than in the intact controls, that most of the follicles were small antral ones (<500 μ in diameter), and that the ratio of normal to atretic follicles in this group (40-50%) was similar to that in the intact rats. All antral follicles of >500 μ in diameter had signs of atresia and appeared to be left over from the previous estrous cycle. As shown in Table 3, the proportion of such follicles in the fasted rats amounted to 8.8% of the total population of growing follicles *vs.* only 1.5% in the intact controls. Chronic undernourishment had thus reduced the total number of growing follicles,

TABLE 1. Effect of Low-Molecular Peptides (LMP) on Estrous Cycle Duration in Fasted Rats ($M\pm m$)

Group	Cycle duration (days) after indicated LMP doses, mg/kg			
	0.2 (for 5 days)	2 (for 5 days)	5 (for 5 days)	5 (once)
Intact (n=10)	4.4±0.2*,**	4.2±0.2*,**	4.2±0.2*	4.3±0.3*
Fasted+given saline (n=11)	9.5±1.3	9.7±1.3	9.9±1.2	9.6±1.5
Fasted+given LMP (n=11)	9.4±1.7	8.1±1.3	5.2±1.3*	6.2±1.2*
Intact+given LMP (n=10)	4.3±0.2*,**	4.2±0.2*,**	4.3±0.2*	4.2±0.3*

Note. Here and in Tables 2 and 3: *$p<0.05$ in comparison with the "fasted+given saline" group; **$p<0.05$ in comparison with the "fasted+given LMP" group.

TABLE 2. Effect of Low-Molecular Peptides (LMP) on the Weight of Reproductive Organs and the Number of Ovulated Oocytes in Fasted Rats ($M\pm m$)

Group	Body weight, g	Uterine weight, mg	Ovarian weight, mg	Number of oocytes
Intact (n=7)	226±7*,**	423±26*	39.8±1.3*	10.9±0.9*
Fasted+given saline (n=7)	173±11	276±16	23.5±1.8	8.3±0.6
Fasted+given LMP (n=7)	201±8	354±18*	32.8±2.8*	10.8±0.9*
Intact+given LMP (n=8)	228±8*,**	430±22*	40.1±1.4*	10.8±0.8*

TABLE 3. Effect of Low-Molecular Peptides (LMP) on the Growth of Antral Follicles in Ovaries of Fasted Rats (*M*±*m*)

Group	Number of antral follicles per ovary			
	normal	atretic, <500	atretic, >500	total
Intact (*n*=6)	83.8±10.4*,**	95.0±9.5*	2.8±0.5*,**	181.0±11.8*
	(45.9±4.5)	(52.5±4.7)	(1.5±0.3)	(100)
Fasted+given saline (*n*=8)	42.1±5.6	54.0±4.8	8.8±0.5	104.9±10.4
	(39.4±2.5)	(51.8±2.0)	(8.8±1.4)	(100)
Fasted+given LMP (*n*=5)	64.4±3.2*	87.6±9.3*	1.8±0.5*	157.8±14.0
	(41.6±2.3)	(56.3±1.9)	(1.1±0.2)	(100)
Intact+given LMP (*n*=6)	90.4±11.2*,**	103.6±9.3*	2.5±0.5*,**	194.2±10.0
	(44.6±4.3)	(53.3±4.9)	(1.5±0.2)	(100)

Note. Figures in parentheses are percentages; atretic <500 and atretic >500 are atretic antral follicles less or more than 500 μ in diameter, respectively.

delayed their growth (prolonged the cycle), but had little effect on the ratio of normal to atretic follicles.

In the fasted rats treated with LMP, the proportion of small antral follicles (<500 μ) was only slightly higher than in the intact controls, while that of large (>500 μ) atretic follicles was even lower; the ratio of normal to atretic follicles was similar in both groups. This indicates that the LMP elevated the overall level of gonadotropins rather than enhancing their binding to follicular receptors, probably because the peptides used lack the capacity to produce paracrine effects.

Depressed reproductive function in the face of reduced food consumption is an appropriate adaptive response of the body to energy deficiency. A loss of 10% to 30% of body weight is critical for the reproductive function in mammals. For example, chronic anestrus was observed in cows fed a diet that caused a 25% loss of body weight [9] and menstrual disorders developed in women whose weight was 10-15% below the ideal value, while a 30% weight loss invariably led to amenorrhea [2].

The inhibition of reproductive function due to food deprivation is known to occur at the central level. The amounts of follicle-stimulating and luteinizing hormones released from the pituitary decrease, as does the frequency of their release, and their ratio also changes. Administration of gonadotropin releasing hormone to starving animals induces the release of luteinizing hormone [2]. All this indicates that the inhibition of reproductive function brought about by undernourishment is associated with hypothalamic dysfunction. Moreover, the resulting hypoglycemia leads to other alterations in the endocrine system, manifested, for example, in lowered levels of insulin and insulin-like growth factor I and elevated concentrations of growth hormone and glucocorticoids in the blood [1,3,8]. Whether metabolic or endocrine changes (or both) are responsible for inhibiting the gonadotropic function of the hypothalamus remains to be elucidated. However, as the results of our study suggest, the ovaries contain species-nonspecific LMP capable of abolishing the effect of nutritional deficiency on reproduction.

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