Biphasic Effects of Antiserum to HCG- plus Gonadotropin-Enhancing Factor(s) on HCG Response of the Immature Mouse (36456)

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Potentiation of gonadal stimulation by luteinizing gonadotropic hormones [luteinizing hormone (LH) and human chorionic gonadotropin (HCG)] has been shown to be effected by gonadotropin-enhancing factor(s) (GEF) extracted from rat or bovine cerebral tissue (1, 2). Thus, gonadal responses were greatly enhanced when male or female rats or female mice were administered microgram quantities of GEF, either in mixture with the hormone or by itself at a time coinciding with release of endogenous LH (1–4). On the basis of results of these and correlative studies, it was proposed that gonadotropin enhancement occurred as a result of molecular interaction between GEF and the gonadotropin (4). This hypothesis was supported by radioimmunological data which showed that the binding affinity of HCG or LH to anti-LH serum was enhanced as much as 50-75-fold and 20- to 50-fold, respectively (5). The postulated (4) GEF-initiated structural change of the HCG molecule which results in increased biological and immunological activity has been studied through use of antisera produced to GEF alone and in mixture with HCG. The present communication reports the effects of antisera to gonadotropin-enhancing cerebral extracts and/or gonadotropic hormones administered with HCG upon gonadal responses in immature mice.

Materials and Methods. Antisera. Antisera were prepared by repeated subcutaneous injection of adult male rabbits with 2000 IU HCG[1] and/or 2.0 mg GEF in 2.1 ml 0.9% sodium chloride solution (saline) homogenized with 2.0 ml complete Freund's adjuvant. Three inoculations were given at 3-week intervals, and the first blood was obtained approximately 3 weeks after the last injection. Thereafter, the antigen was injected at approximately 3-month intervals. Blood was collected by cardiac puncture, and the serum obtained following centrifugation at 2000*g* for 30 min was stored in 0.5-ml aliquots at —20° with sodium azide added (0.1%) as a preservative.

Anti-LH serum[2] was prepared by inoculating male rabbits with sheep LH (NIH-LH-S11) mixed with Freund's adjuvant.

Heated serum. Freshly thawed serum, at full concentration or diluted 1:5 with saline, was heated 60 min in a water bath maintained at 65°. Denatured protein was removed following centrifugation of the serum for 30 min at 2000*g*, 4°. In two experiments sera at full concentration were maintained at 92–95° for 1 hr, and the resulting coagulated mass was dispersed by homogenization in saline prior to centrifugation as described above.

Gonadotropin assay. The capacity of HCG to induce growth of the immature mouse uterus was employed as an index of gonadotropin activity. Mice of the CD-1 strain (Charles River Breeding Laboratories) weighing 10–12 g at 21 days of age were used when they were 24 days old. The total dose of HCG, with or without added serum diluted with saline, was injected sc in three 0.2-ml volumes on 3 consecutive days. The largest total volume of AS, anti-HCG serum (1-H) and normal rabbit sera (NRS) was 5 μl, as used for the 1:120 dilution. Twenty-four hours after the last injection, the animals

[1] A. P. L., Ayerst Laboratories, Inc., New York.

[2] Generously donated by Dr. Bruce Goldman, Department of Biobehavioral Sciences, University of Connecticut.

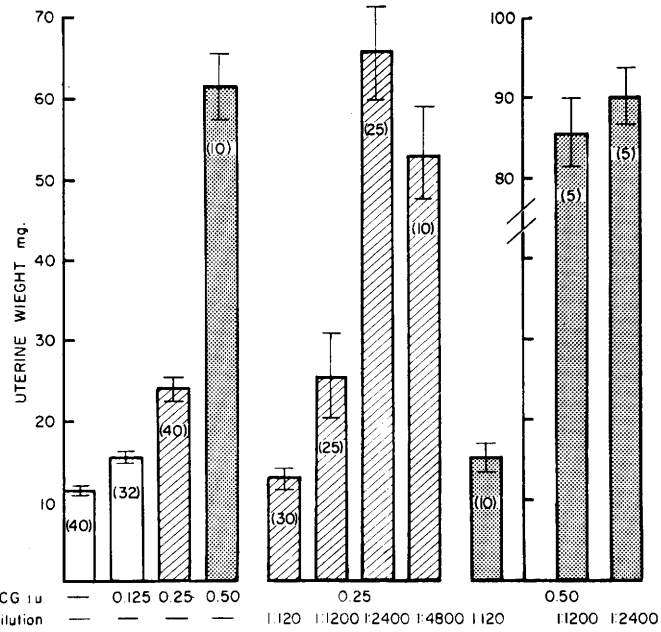

FIG. 1. Uterine-weight responses to HCG standards (left) modified through use of AS diluted with saline as the solvent for injection in mice. Responses to standards dissolved in saline are depicted with bars cross-hatched (0.25 IU) or stippled (0.50 IU) corresponding to the markings of the inhibited and potentiated weights produced by 0.25 and 0.50 IU HCG, shown in the center and right groups, respectively. Values presented are the mean ± SEM of the number of animals indicated in parentheses.

were sacrificed under ether, and uteri were rapidly removed, lightly blotted and weighed to the nearest 0.05 mg on a micro-torsion balance.

Preparation of GEF. The cerebral fraction (RCF-22-3) used in these studies was prepared as described earlier (4) by fractional precipitation from 80%-saturated ammonium sulfate.

Results. Biphasic effects of anti-HCG/GEF serum (AS) on HCG activity. A biphasic response was obtained in immature mice administered 0.25 or 0.50 IU of HCG dissolved in AS at several different dilutions (Fig. 1). The mean uterine weight of mice treated with 0.25 IU HCG mixed with AS diluted 1:120 was significantly less than those obtained following injection of HCG alone ($p < .001$)[3,4]. The uterotropic response to 0.50, 1.00 and 1.50 IU HCG (see below) were also inhibited significantly by mixture with AS 1:120 ($p < 0.001$). The antiserum-inhibited responses to these four doses of HCG did not differ significantly from one another or from the mean uterine weight of mice which received only saline.

The HCG-inhibitory effect was reversed when the serum was diluted 1:2400 or 1:4800 for administration in mixture with HCG (Fig. 1). The uterine response to 0.25 IU HCG in AS-1 diluted 1:2400 was as great as the response to 0.50 IU HCG in saline. Hormonal enhancement began to weaken when AS was diluted 1:4800. The uterine response to 0.50 IU HCG was similarly increased by the serum diluted 1:1200 and 1:2400 ($p < 0.01$ and $p < .001$, respectively) (Fig. 1).

Comparative effects of rabbit sera on HCG activity. Three different preparations of antisera (AS, anti-LH and 1-H) were compared to NRS in mixture with HCG (Fig. 2). Anti-HCG serum inhibited the gonadal response to 0.25 IU HCG when diluted as

[3] Mean ± SEM.

[4] Statistical analysis performed by Student's t test.

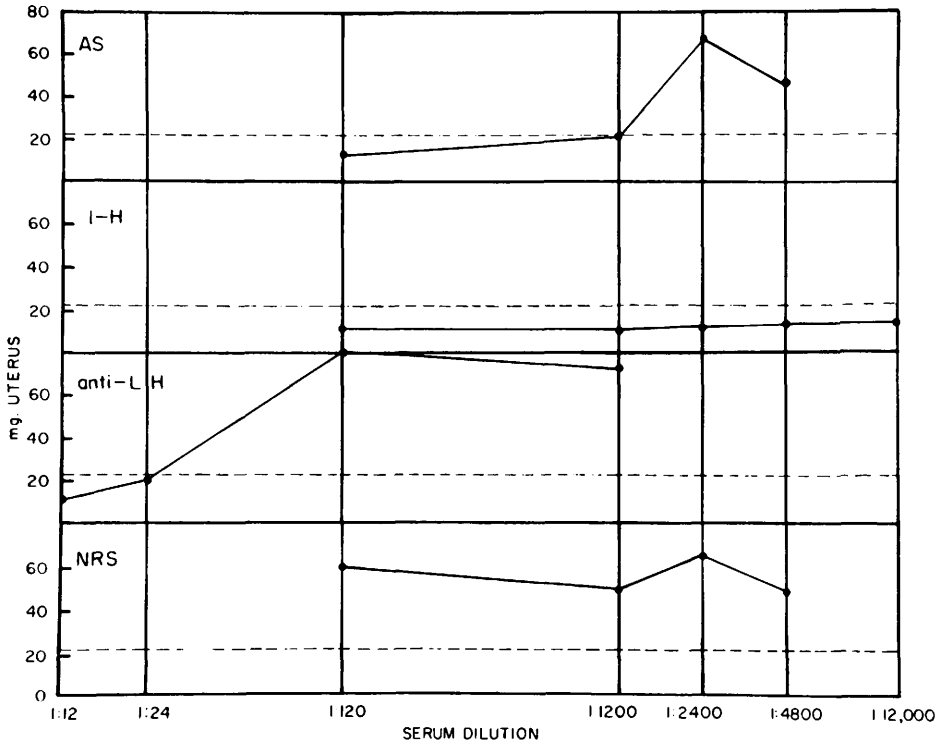

FIG. 2. Biphasic effects of AS used as diluent for the hormone on the response to 0.25 IU HCG, compared to the influence of 1-H, anti-LH and normal rabbit sera similarly employed. See text for details of preparation of sera. The mean response of 64 mice to 0.25 IU HCG injected in saline was 23.0 ± 1.1 mg (- -).

much as 1:12,000. NRS potentiated the uterine response at all dilutions tested, 1:120 to 1:4800. Anti-LH serum inhibited HCG only insignificantly at a 1:12 dilution (Fig. 2, Table II), and a striking enhancement of hormonal effectiveness was elected by mixture with the serum diluted 1:120 and 1:1200.

Effect of heat on HCG-inhibitory activity. HCG-inhibitory activity of AS and 1-H was stable to heating at 65° for 60 min (Table I). Resistance of the anti-HCG/GEF preparation to heat was explored further with 5 mice/group receiving AS in combination with doses of HCG up to 2.00 IU. AS significantly inhibited the uterotropic response to 0.50, 1.00 and 1.50 IU HCG, with sera heated at 65° or unheated equally effective in combination with each dose of hormone ($p < .001$, $< .005$, and $< .0001$, respectively). It was noted that the mean uterine weight of mice which received 1.50 IU (80.3 ± 2.5) or 2.00 IU HCG (68.3 ± 4.2) was significantly less than that attained by mice treated with 1.00 IU (94.4 ± 9.1; $p < .05$). The apparent decrease in sensitivity to higher doses of HCG was reversed when AS (1:120) mixed with 2.00 IU *enhanced* the depressed response (87.3 ± 6.3; $p < .05$ compared to 68.3 ± 4.2 mg).

When AS and 1-H were heated at full concentration at 92–95°for 60 min, the capacity to inhibit HCG activity at a 1:120 dilution was lost (Table II). On the contrary, the heated antisera significantly enhanced 0.25 IU HCG. However, the strongly inhibitory anti-HCG serum diluted 1:2400 following heat treatment did not enhance the HCG response.

Discussion. The antibodies of AS which block the uterotropic response to HCG possess relatively great resistance to heat denaturation. For, it is known that heating serum for 30 min at 56° does not destroy the activi-

TABLE I. Effect of 65° Heat on HCG-Inhibiting Activity of Antisera.[a]

Treatment	No. of animals	Uterine wt (mg ± SEM)
Saline	15	13.4 ± 1.0
0.25 IU HCG	15	21.5 ± 2.2
0.25 IU HCG plus:		
AS, 1:120	16	12.5 ± 0.9[b]
Δ AS, 1:120	15	14.9 ± 1.3[c]
1-H, 1:120	5	8.9 ± 1.1[b]
Δ 1-H, 1:120	5	11.1 ± 0.7[b]
anti-LH, 1:24	5	20.1 ± 2.0
Δ anti-LH, 1:24	5	30.1 ± 2.9

[a] Heated samples (Δ) were incubated at 65° for 60 min.

[b] p vs 0.25 IU HCG, $<.001$.

[c] p vs 0.25 IU HCG, $<.02$.

ty of antibodies (6), although certain antibodies stable at 56° for as long as 24 hr endure 60° heat for a much shorter period of time (7). Nevertheless, all of the antisera, when heated at 92–95° for 60 min at full concentration, failed to inhibit the HCG response at the respective concentrations ordinarily inhibitory. These more intensely heated sera each displayed heat-stable HCG-enhancing potency which appears to be intrinsic to the serum [cf. (8)]. The failure of 1-H serum diluted 1:2400 to display enhancing activity following 92–95° heating probably indicates that its limited content of GEF had been effectively "diluted-out."

The biphasic influence upon HCG response obtained with graded dilutions of AS and, to a less marked degree, anti-LH serum, is consistent with the hypothesis that the inhibitory components are titrated against the facilitatory activity of the antisera at any given dilution and that the inhibitory factors are dominant only at higher serum concentrations.

Other investigators have described the presence consecutively of gonadotropin-enhancing and -inhibiting activities in sera of animals inoculated with pituitary extracts or gonadotropic hormones. Thompson (9) reported the occurrence of FSH-augmentor followed by anti-FSH activity in sera of a female dog and horse immunized with pituitary extracts. In the studies of Marvin and Meyer (10), equine serum similarly prepared antagonized the effects of HCG, PMS and pituitary extracts from a variety of species. Snook and Cole (11, 12) found both FSH-like and FSH-potentiating activities in serum following the rise and decline of anti-HCG titers in mares chronically treated with HCG.

The dual activity of anti-HCG/GEF sera reported here differs in several respects from that of sera described above. GEF potenti-

TABLE II. Effect of 92–95° Heat on HCG-Inhibiting Activity of Antisera.[a]

Treatment	No. of animals	Uterine wt (mg ± SEM)	p[b]
Saline	5	12.8 ± 2.0	
0.25 IU HCG	10	18.7 ± 2.8	
0.25 IU HCG plus:			
AS, 1:120	5	10.6 ± 1.1	$<.02$
Δ AS, 1:120	5	45.6 ± 12.2	$=.05$ ($=.02$)
anti-LH, 1:12	6	12.6 ± 1.7	NS
Δ anti-LH, 1:12	6	54.2 ± 14.1	$<.05$ ($<.02$)
1-H, 1:120	5	14.1 ± 1.2	NS
Δ 1-H, 1:120	5	57.1 ± 4.0	$<.001$ ($<.001$)
1-H, 1:2400	5	10.4 ± 1.0	$<.02$
Δ 1-H, 1:2400	5	14.4 ± 0.4	NS (NS)

[a] Heated samples (Δ) were incubated at 92–95° for 60 min.

[b] Probability of significance of the difference between HCG response with and without antiserum. p values in parentheses indicate statistical comparison was made between heated and unheated antisera.

ates responses to the luteinizing gonadotropins, but not to FSH (4). GEF alone has repeatedly failed to display LH-like activity in acute studies, although the weight of gonads and sex accessory glands and body weight tended to be increased in male rats chronically injected with GEF (4). Moreover, GEF does not bind to anti-human-LH serum (5, 12).

The nature of the suppression of HCG and/or GEF by AS is being explored in studies currently in progress which employ an agar diffusion technique. Evidence is being sought for a molecular interaction between antibody and HCG/GEF in mixture which is distinct from the reaction between antibody and GEF or antibody and HCG. The latter distinction, if established, would provide further support for the hypothesis (4) that GEF interacts directly with the HCG molecule to enhance its immunological as well as biological activity. Such a demonstration might also increase understanding of the dynamics of the gonadotropin-enhancing and gonadotropin inhibiting factors present in the antisera studied here.

Summary. Antisera prepared to HCG/GEF (abbreviated AS) inhibited the uterotropic response of immature mice when HCG was assayed in sera diluted 1:120. The response to HCG dissolved in AS diluted 1:2400 or 1:4800 was enhanced. Enhancement was also produced by normal rabbit serum similarly employed at all dilutions tested, 1:120 to 1:4800. Anti-HCG serum, powerfully inhibitory at all dilutions tested as a solvent for the hormone, revealed enhancing activity when subjected to 60 min of heat at 92–95°. The latter treatment also abolished HCG antagonism by AS, although the respective antibodies of both the latter and the former sera were stable to treatment at 65° for 1 hr. These combined data suggest that each of the antisera studied contained not only their respective antibody species, which inhibited the response to HCG, but also possessed some degree of gonadotropin-enhancing potency. Thus, the results obtained can be interpreted as the resultant of superimposition of an inhibitory influence upon enhancing activity, both present simultaneously in the sera.

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