Inhibin Production by Sertoli Cells During Testicular Regression in the Golden Hamster

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The objective of this study was to determine if testicular regression in the hamster results in changes in Sertoli cell function that reflect altered pituitary function. Inhibin-like activity was measured in spent media from Sertoli cells cultured from adult control and optically enucleated hamsters with regressed testes using a homologous hamster Sertoli cell/hamster pituitary cell bioassay. The inhibin activity resulted in a dose-related decrease in FSH release from both normal and "regressed" pituitary cultures but maximal inhibition occurred at a 3- to 4-fold lower dose with media from Sertoli cells obtained from regressed hamsters. When pituitary cells from control adult or adult hamsters with regressed testes were incubated with Sertoli cell spent media, pituitary cells from regressed hamsters were more sensitive than normal pituitary cells to both normal and "regressed" inhibin. This greater production of inhibin-like activity and/or an enhanced sensitivity to inhibin in the regressed hamster may contribute to the decline in FSH levels during testicular regression. This data lends further support to a physiologic role for inhibin.

Key words: Sertoli cell, hamster, inhibin, pineal, testis.

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Inhibin is defined as a non-steroidal substance of gonadal origin that selectively regulates FSH secretion (Franchimont et al, 1979). It has been found in both sexes: in the ovarian follicular fluid from the female (Grady et al, 1982; Channing et al, 1985) and in testis extracts, seminal fluid, and Sertoli cell-conditioned medium from the male (Steinberger, 1981; De Jong and Robertson, 1985). It has also been detected in numerous species including man (Channing et al, 1984, 1985; Chari et al, 1985), rat (Erickson and Hsueh, 1978; LeGac and de Kretser, 1982; Steinberger et al, 1983; Verhoeven and Franchimont, 1983; Au et al, 1984; Croze and Franchimont, 1984), hamster (Chappel, 1979), cow (Henderson and Franchimont, 1983), rabbit (Goodman, 1984), ewe (McNeilly, 1984), pig (Lepner and Dhanarajan, 1984), sheep (Vijayalakshmi et al, 1980), and monkey (Hoover et al, 1983).

There are now numerous examples in both sexes of the possible physiologic importance of inhibin. For example, follicular fluid containing inhibin can suppress serum FSH in intact rats and also selectively inhibit the rise in serum FSH observed after castration in several species (Channing et al, 1985). In the male cryptorchid rat, an inverse relationship exists between serum FSH and both testicular content of inhibin and the rate of inhibin accumulation in the

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testis following efferent duct ligation (Au et al, 1984). These results provide indirect evidence for the hypothesis that inhibin plays an important physiologic role in the control of FSH secretion. However, the true physiologic significance of inhibin is yet to be established (Channing et al, 1985). To obtain a clearer picture of the physiologic role of inhibin, it is necessary to have a model system in which FSH levels are physiologically modulated and to correlate causally these changes to alterations in inhibin production and action.

The photoperiodic hamster may be a good model system for further elucidating the physiologic role of inhibin in the feedback regulation of FSH secretion. The golden hamster is a seasonal breeder. Exposure to less than 12.5 hours of light per 24 hours results in a decline in serum LH, FSH, prolactin and testosterone levels that is followed by a decline in serum LH, FSH, prolactin and testosterone levels that is followed by a decline in spermatogenesis, ultimately resulting in testicular regression (Reiter, 1980; Bartke et al, 1980). This pineal gland-mediated atrophy of the reproductive system of the male hamster occurs slowly over a period of 10 to 12 weeks, is maintained for 2 to 4 weeks, and is followed by a spontaneous recrudescence of the reproductive system. This recrudescence (which is complete by 20 to 25 weeks after placement into the short photoperiod or optic enucleation) is characterized by an increase in serum levels of LH and FSH that reach a peak several times normal adult levels and then level off at adult levels, followed by a reinitiation of spermatogenesis and normal testicular and sex accessory organ weights (Reiter, 1980; Berkowitz and Heindel, 1984).

Since inhibin has been shown to be produced by Sertoli cells and Sertoli cells continue to produce inhibin in culture (Steinberger & Steinberger, 1976; Au et al, 1985), we have utilized Sertoli cell cultures to ascertain if there is a correlation between testicular regression and inhibin production in the hamster. The results of this study indicate that inhibin production does indeed mirror the FSH serum levels during pineal-mediated testicular regression and, therefore, the hamster appears to be an appropriate species to utilize in the assessment of the physiologic significance of inhibin.

Materials and Methods

Hormones, Isotopes, and Chemicals

Ovine FSH ((NIH FSH-14), rat FSH RP-1 and LH RP-1 RIA standards, rat FSH I-4 hormone for iodination, and rat anti-FSH serum S-10 were provided by the NIADDK Pituitary Hormone Distribution Program. Ovine LH (LER 1056 C2) for iodination was supplied by Dr. Leo E. Reichert, and antiovine LH serum (GDN 15) was obtained from Dr. Gordon D. Niswender. Goat antirabbit gammaglobulin was purchased from Antibodies, Inc. (Davis, CA). DNase was purchased from Sigma Chemical Co. (St. Louis, MO). Trypsin, Eagle's Minimum Essential Medium with Earle's Salts and essential amino acids (MEM), penicillin-streptomycin, fungizone, and MEM vitamins were purchased from Grand Island Biological Co. (GIBCO, Grand Island, NY). Collagenase was purchased from Worthington Diagnostics (Freehold, NJ). Carrier-free [^{125}I]Na was purchased from Amersham (Arlington Heights, IL). All other chemicals were of reagent grade quality.

Animals

Hamsters were obtained from Engle Laboratory Animals (Farmersburg, IN) or from a randomly bred colony based on Engle stock maintained in our research laboratories. Animals were housed in a photoperiodically regulated facility (14 hours of light, 10 hours of darkness; lights on from 0500 to 1900 hours) and were provided with food and water *ad libitum*. Optic enucleation and ear tagging (under Metofane anesthesia) were carried out at approximately 9 to 10 weeks of age. Adult (control) hamsters (20 to 22 weeks of age) or optically enucleated hamsters 12 weeks postenucleation (3 to 5 animals pooled per experiment) were sacrificed by decapitation, and the trunk blood was collected and stored at −20 C for RIA of gonadotropins. Testes were removed under aseptic conditions, weighed, and decapsulated. The decapsulated testicular weight was determined for each hamster.

Sertoli Cell Isolation and Culture

Sertoli cells were isolated from either normal adult hamsters (20 to 22 weeks of age) or adult hamsters optically enucleated 12 weeks earlier to induce testicular regression according to the procedures previously described (Berkowitz and Heindel, 1984). Sertoli cells were cultured in 100-mm culture dishes at 0.05 testis equivalent/dish in 5 ml MEM containing penicillin-streptomycin-fungizone and MEM vitamins at 34 C under an atmosphere of 5% CO_2/95% air and 95% relative humidity. After 3 days, media were removed, discarded, and fresh media added. After an additional 4 days, the media were removed and saved for inhibin bioassay.

Pituitary Cell Isolation and Culture

Adult male hamsters or rats (10 to 15 per culture) were decapitated and the anterior lobes of the pituitaries were aseptically removed and placed in Ca++- and Mg++-free Hanks Balanced Salt Solution (HBSS; GIBCO). The pituitaries were minced and incubated at 37 C in a trypsinizing flask with 0.1% collagenase in Hanks Balanced Salt Solution (1 ml/pituitary; Worthington). The pituitaries were incubated with constant shaking (100 shakes/minute), and at 10-minute intervals were triturated 10 to 15 times against the side of the flask. After 40 minutes of incuba-

tion, 0.2% DNase (10 μl/ml Hanks Balanced Salt Solution: Sigma) was added, and incubation was resumed for 5 to 8 minutes. After 45 to 48 minutes total incubation time, the dissociated cells in Hanks Balanced Salt Solution were transferred to a sterile 50-ml conical tube and centrifuged at 200 × g to pellet the cells. The supernatant was decanted, and the pellet resuspended in 2 ml fresh MEM. Cell density was determined by multiple countings in a hemocytometer. Cells were diluted to 1×10^5 cells/ml and plated into 16-mm multi-well culture plates (Costar) at 1 ml/well. Medium for pituitary cells was similar to that prepared for Sertoli cell cultures, with the addition of 15% horse serum and 2.5% fetal bovine serum. Incubation was at 37 C for four days under an atmosphere of 5% CO_2/95% air and 95% relative humidity.

Inhibin Bioassay

After 4 days of culture at 37 C, media were removed from the pituitary cultures, the cultures were rinsed and incubated for 3 additional days with 1 ml of media containing various volumes (1000 μl, 500 μl, 250 μl) of Sertoli cell spent media from control adult Sertoli cell cultures or Sertoli cell cultures of regressed hamsters. Media were removed and hamster or rat LH and FSH was measured according to procedures outlined previously (Berkowitz and Heindel, 1984). All results were normalized to 100,000 pituitary cells.

Pituitary Sensitivity Assay

Pituitary cultures from normal adult hamsters or adult hamsters with regressed testes were prepared and cultured as described above. After 4 days of incubation, the medium was removed from each culture and replaced by Sertoli cell spent media from control adult cultures or from adult hamsters with regressed testes. After 72 hours, the media were removed, centrifuged to remove cells and frozen for later assay of FSH release.

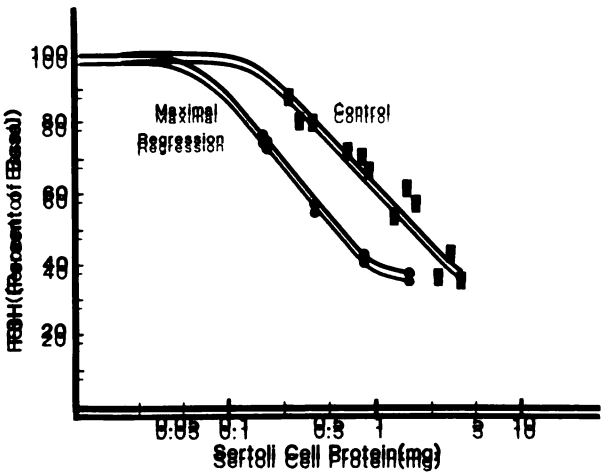

Fig. 1. Dose response curve of inhibition of basal FSH release by Sertoli cell spent media from control adult and adult optically enucleated hamsters with regressed testes. The control curve is pooled data from three separate experiments and the maximal regression curve is pooled data from two separate experiments. Each experiment consisted of a Sertoli cell culture prepared from a pool of three to five hamsters. Each point is the average of duplicate determinations. Data are plotted as Sertoli cell protein equivalent/volume of media assayed. Average basal release of FSH was 1024 ± 59 ng FSH/10^5 pituitary cells/72 hours for all cultures.

TABLE 1. Inhibin Production by Cultured Sertoli Cells from Adult Hamsters: Specificity of Response*

Source of Media	FSH (ng/10^5) cells ± SEM)	
	Rat Pituitary	Hamster Pituitary
Control	67 ± 8 (a)	1024 ± 59 (c)
Hamster Sertoli cells	69 ± 8 (a)	420 ± 60 (d)
Rat Sertoli cells	41 ± 4 (b)	-

*Hamster or rat pituitary cells were prepared as described in the Methods section and incubated for 96 hours in the presence of 1 ml of spent media (1.8 mg protein/dish/5 ml media) from 4-day cultures of either hamster or rat Sertoli cells. Each value is the average of quadruplicate determinations ± SEM. Values with different letters are significantly different at the $P < 0.05$ level as determined with ANOVA.

Results

Since most of the data concerning inhibin production have been obtained using rat pituitary cultures as a bioassay, we initially tested cultures of Sertoli cells from adult hamsters for inhibin production using a rat pituitary culture. Table 1 shows that this rat bioassay system was responsive to spent media from rat Sertoli cell cultures but not to that from hamsters. When the homologous hamster/hamster bioassay system was utilized, the presence of inhibin in spent media from hamster Sertoli cells could be detected. This homologous bioassay system was then used to determine the effect of pineal-induced testicular regression on inhibin production by cultured Sertoli cells. Figure 1 shows that, when expressed per mg Sertoli cell protein, spent media from Sertoli cell cultures of normal adult hamsters (average testis weight: 1620 mg) produced a linear dose-related inhibition of FSH release. When spent media from cultures of Sertoli cells obtained from regressed hamsters (average testis weight: 262 mg) was tested, a linear dose-related inhibition of FSH release parallel to that of normal adult hamster Sertoli cell cultures was obtained. Fifty percent inhibition occurred at 0.60 mg protein from "regressed" cultures compared with 2.1 mg protein for normal adult hamsters. A small (less than 25%) and non-dose-related effect of Sertoli cell spent media on pituitary cell LH release

also was detected in these studies (Table 2). A comparison of the data in Table 2 and Fig. 1 reveals that the medium from a control Sertoli cell culture containing 1.8 mg Sertoli cell protein had no significant effect on LH release in the bioassay while inhibiting FSH release 50%. Medium from a Sertoli cell culture from hamsters with a regressed testis had a significant (25%) but non-dose-related inhibition (same effect over a 10-fold dilution of Sertoli cell protein) of LH release while this same medium caused a dose-related inhibition of FSH release of from 60 to 15%. The average serum FSH concentration for the control animals was 208 ± 30 ng/ml while that of the regressed animals was 78 ± 20 ng/ml (N = 4).

An alteration in the pituitary sensitivity to inhibin also occurred during testicular regression (Fig. 2). The pituitary cultures from the regressed hamsters showed a greater inhibition of FSH release than pituitary cultures from normal adult hamsters whether the spent media were from normal control adult Sertoli cell cultures or from Sertoli cell cultures from regressed hamsters. FSH release from control pituitaries was inhibited 60% compared with the 80% inhibition of FSH release from the pituitaries of regressed animals regardless of the source of the spent media.

Discussion

These results show that "inhibin" is produced by hamster Sertoli cells as measured by a homologous hamster/hamster bioassay. The fact that the heterologous hamster/rat bioassay system did not detect inhibin activity indicates either a large difference in the sensitivity of the assay systems (i.e. hamster pituitaries are exquisitely more sensitive to inhibin) or that there is species specificity in the inhibin produced. Heterologous inhibin assays for porcine/rabbit, porcine/rat, and ovine/rat have been reported (Jenner et al, 1982; Goodman, 1984; Channing et al, 1985). However, Channing et al (1985) also reported in a recent review that it was difficult to ascertain whether inhibin preparations derived from various species are identical or dissimilar. In fact, they reported differences in behavior of porcine and bovine ovarian inhibin on Matrix gel Red A. Our data lend support to the hypothesis of species specificity of inhibin.

The linearity of the FSH dose inhibition curves for hamster Sertoli cell inhibin, along with the data showing that there is no significant dose-related effect on LH release, validates this homologous bio-

TABLE 2. Effect of Sertoli Cell Spent Media on LH Release from Normal Pituitary Cultures*

Source of Media		LH Release (ng LH/10^5 cells)
MEM control		1243 ± 55 (a)
Adult control Sertoli cells		
(Sertoli cell mg protein)	1.8	1087 ± 88 (a)
	0.36	1063 ± 41 (a)
Adult "regressed" Sertoli cells		
(Sertoli cell mg protein)	1.7	923 ± 51 (b)
	0.17	1015 ± 72 (b)

*Sertoli cell spent media from adult control cultures or cultures from adult hamsters with regressed testes were incubated with pituitary cultures from normal adult hamsters for 72 hours. Results are the average of quadruplicate determinations ± SE (each in duplicate) from a representative experiment repeated in triplicate. Values are presented as the Sertoli cell protein equivalent (i.e. Sertoli cell protein per dish from which medium was removed and assayed). Values with the same letter are not significantly different by one-way ANOVA analysis. The protein values correspond to the values in Fig. 1.

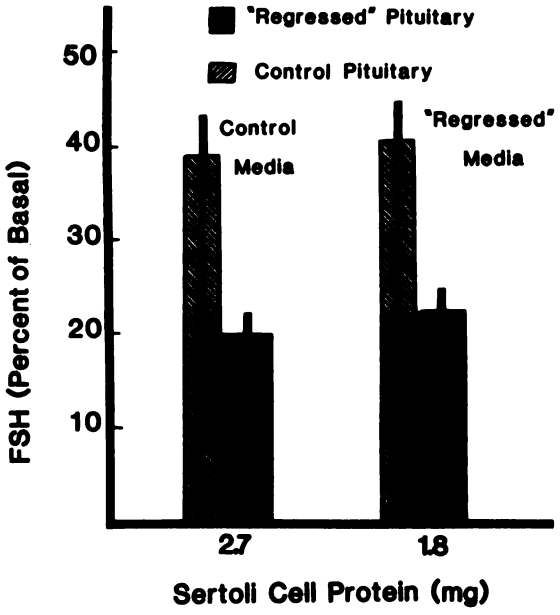

Fig. 2. Effect of source of hamster pituitaries on the "perceived" inhibin activity of Sertoli cell spent media. Spent media obtained from Sertoli cell cultures of normal or regressed testes (from a pool utilized in Fig. 1) at two concentrations were incubated with pituitaries from control or regressed hamsters for 72 hours as described in the Methods section. Each bar is the average ± SE of three control media experiments (N = 12) and two regressed media experiments (N = 8), each bioassayed in quadruplicate in a single pooled pituitary culture.

assay system. It should be noted that the Sertoli cell cultures were prepared by standard techniques that result in cultures virtually free of Leydig cells but with some contaminating germ cells and myoid cells, usually of the order of less than 10% (Dorrington et al, 1975, Welsh and Wiebe, 1975; Steinberger et al, 1979; Chapin and Gray, 1986). We (Wagle et al, 1986) have recently shown that hypotonic shock that removes germ cells has no effect on inhibin activity of the spent media from Sertoli cells, indicating that the contaminating germ cells have no significant effect on inhibin production. Similarly, it has been shown that neither seminiferous tubule myoid cells or germ cells produced inhibin-like activity (Steinberger and Steinberger, 1976). These data validate the use of the Sertoli cell culture system to monitor inhibin production. Nonetheless, since the "inhibin" was measured utilizing a bioassay with an impure preparation of Sertoli cell spent media, the term inhibin activity is used instead of inhibin concentration. This distinction allows for the possibility of the production of a component that is distinct from inhibin but that augments the activity of inhibin.

It is noteworthy that spent media from cultures of Sertoli cells from regressed hamsters are more potent in inhibiting FSH release (i.e. higher inhibin activity) than media from Sertoli cell cultures from normal adult hamsters. These data correlate with the physiologic changes that occur during pineal-induced regression of the reproductive system and suggest the possibility that the increased inhibin activity may be, at least partially, responsible for the decline in serum FSH levels during regression. These data therefore add to the growing body of information indicating a physiologic role for inhibin.

Further indication of the physiologic relevance of these results comes from the data showing increased sensitivity of pituitaries from regressed hamsters to the inhibin activity. The results suggest that pituitaries from regressed hamsters can be inhibited to a greater degree (i.e. 80% vs. 60%) by inhibin activity from cultures of either normal or regressed animals. The increased maximal effect also occurred at a lower dose of inhibin, suggesting a shift both in the sensitivity and maximal effect. This may indicate a multipoint attack on the reproductive system during regression: increased inhibin production and increased pituitary sensitivity to the action of inhibin. Further experiments currently in progress on the time course of the alterations of inhibin production during testicular regression/recrudescence may indeed show a causal relationship between inhibin production and FSH levels, thereby substantiating a physiologic role for inhibin in the golden hamster.

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