Gonadotrophin-inhibiting activity in seminal plasma from intact and vasectomized bulls

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Summary. Bull seminal plasma administered to male rats at the time of castration inhibited the rise in the levels of FSH and LH otherwise seen in the serum 24 h later. That the gonadotrophin-inhibiting activity was also present in the seminal plasma from vasectomized bulls suggests that it was not of testicular origin. Although the substance with gonadotrophin-inhibiting activity was a protein, it may be chemically distinct from inhibin.

Introduction

Inhibin was the name given by McCullagh (1932) to a testicular hormone that would regulate the secretion of follicle-stimulating hormone (FSH) by negative feedback. Various tissues and secretions of the male reproductive tract, including testicular extracts (bovine: Keogh *et al.*, 1976; ovine: Nandini, Lipner & Moudgal, 1976; Moodbidri, Joshi & Sheth, 1976; human: Chari, Hopkinson, Fritze, Sturm & Hirschhauser, 1977; rat: Le Lannou & Chambon, 1977), epididymal extracts (rat: Le Lannou & Chambon, 1977), rete testis fluid (ovine: Setchell & Jacks, 1974; Blanc & Dacheux, 1976; Franchimont *et al.*, 1976; Davies, Main, Young & Setchell, 1976), seminal plasma (bovine: Franchimont, Chari, & Duraiswami, 1975a; human: Franchimont, 1974) and sperm extracts (bovine: Lugaro, Casellato, Mazzola, Fachini & Carrea, 1974), contain FSH-suppressing activity which has been attributed to inhibin. Other evidence for inhibin comes from studies of selective damage to the germinal epithelium in which blood levels of FSH are elevated, while luteinizing hormone (LH) levels are often normal or only slightly elevated (Amatayakul, Ryan, Uozumi & Albert, 1971; Swerdloff, Walsh, Jacobs & Odell, 1971; Debeljuk, Arimura & Schally, 1973; Gomes, Hall, Jain & Boots, 1973; Rich & de Kretser, 1977), and from the secretion of FSH-inhibiting activity from cultured Sertoli cells (Steinberger & Steinberger, 1976).

The above evidence suggests the origin of inhibin and its route to the general circulation. Inhibin would be synthesized in the Sertoli cells, perhaps in conjunction with the spermatogonia or spermatids, would be secreted into the seminiferous tubules and transported in the rete testis fluid to the head of the epididymis, where it would be absorbed along with most of the rete testis fluid (Franchimont, Chari, Hagelstein & Duraiswami, 1975b; Chari, 1977). The presence of FSH-inhibiting activity in seminal plasma could arise from incomplete absorption of inhibin in the epididymis (Franchimont *et al.*, 1975b). We have tested this hypothesis by measuring the amount of FSH-inhibiting activity in seminal plasma from intact and vasectomized bulls. A preliminary account of this work has been presented (Peek & Watkins, 1978).

Materials and Methods

Bull seminal plasma was obtained from the New Zealand Dairy Board Artificial Breeding Centre, Newstead, Hamilton, and was stored at -20°C until use. The seminal plasma from intact bulls was a pool of samples from 20 animals of good fertility. The seminal plasma from vasectomized bulls was a pooled sample from 5 bulls vasectomized over 1 year earlier. Indivi-

dual samples of seminal plasma were obtained from another 4 vasectomized bulls. All vasectomized animals were of proven fertility before vasectomy, but were not used for breeding because of genetic reasons.

Protein was precipitated from seminal plasma at 4°C by the addition of absolute ethanol to a concentration of 86% (v/v) (Franchimont *et al.*, 1975a). The precipitate was removed by centrifugation at 3000 g for 10 min and freeze-dried. This procedure gave 80 and 46 mg pale yellow powder/ml seminal plasma from intact and vasectomized bulls respectively.

Solutions of the seminal plasma extracts (I from intact and V from vasectomized bulls) were heated and digested with pepsin to denature the protein. The heat treatment for 400 mg Extract I and 230 mg Extract V consisted of dissolution in 10 ml saline (9 g NaCl/l) and heating in a water bath at 67°C for 1 h. The solutions were then snap-frozen and stored at -20°C. For pepsin digestion, 400 mg Extract I or 230 mg Extract V were dissolved in 10 ml 0.2 M-glycine-HCl buffered at pH 2, and 1 mg pepsin (Sigma Chemical Co., St. Louis, Missouri) was added per 50 mg extract. The solutions were incubated at 37°C for 24 h, the pH was then brought to 7.5 with 2 M-sodium hydroxide, and the solutions were snap-frozen and stored at -20°C.

FSH- and LH-inhibiting activity were detected by the suppression of the post-castration rise of serum levels of FSH and LH in (1) pubertal rats (Nandini *et al.*, 1976; Davies *et al.*, 1976) or (2) chronically castrated adult rats (Setchell & Jacks, 1974; Franchimont *et al.*, 1975a). For Test 1, 5-week-old Sprague-Dawley rats were castrated through a scrotal incision, under penthrane-nitrous oxide anaesthesia. The material to be assayed was administered in a single intraperitoneal injection while the animal was still anaesthetized. Seminal plasma samples were diluted to 0.5 ml with saline; Extracts I and V were dissolved in 0.5 or 1.0 ml saline while the heated and pepsin-treated extracts were injected undiluted. The rats were bled after decapitation 23–25 h later.

For Test 2, Sprague-Dawley rats weighing 200–300 g were castrated 3 weeks before treatment, which consisted of daily intraperitoneal injections of up to 1.0 ml seminal plasma from intact bulls for 2–4 days. The rats were bled at decapitation 4 or 24 h after the last injection.

FSH and LH concentrations in 100 µl and 200 µl aliquots of serum were measured by radioimmunoassay using the reagents and method supplied by the NIAMD Rat Hormone Program. The sensitivity of the LH and FSH assays were 4 ng LH-RP1 and 30 ng FSH-RP1 respectively. Samples with LH concentrations below 20 ng/ml were assigned a concentration of 20 ng/ml for statistical analysis. The interassay coefficient of variation was 10.9% for FSH and 12.0% for LH, while the average intra-assay coefficient of variation was 6.3% for FSH and 5.3% for LH. To reduce error, samples from a single experiment were assayed together.

Hormone concentrations were assumed to be log-normally distributed. The one-tailed Student's *t* test was applied to the logarithmically transformed data to detect whether treatment groups had means significantly lower than the mean of the control groups receiving saline.

Results

When seminal plasma from intact bulls was administered to male rats at the time of castration (Test 1), the rise in serum concentrations of FSH and LH otherwise seen 24 h later was inhibited. The degree of inhibition was clearly related to the amount of seminal plasma given (Table 1a). At a dose of 0.5 ml, FSH and LH levels were always significantly lower than those of the control group receiving saline; for FSH, $P < 0.005$ in all of 14 separate experiments, and for LH, $P < 0.05$ in all of the 14 experiments and $P < 0.005$ in 10 of the 14 experiments. Rats receiving 0.5 ml seminal plasma usually had FSH and LH concentrations comparable to those of intact animals. At a dose of 0.2 ml, FSH levels were significantly ($P < 0.05$) lowered in 3 of 5 experiments, and LH levels in 2 of 5 experiments. In contrast, the rats in Test 2, castrated 3 weeks before the onset of treatment with seminal plasma from intact bulls, did not show any lowering of FSH or LH levels.

Seminal plasma from vasectomized bulls also inhibited the post-castration rise of FSH and LH levels in Test 1 (Table 1b) and, moreover, to the same extent as did the seminal plasma from intact bulls. A 0.5 ml volume of the pooled or individual samples always significantly ($P < 0.05$) suppressed FSH and LH values near to those of intact rats, while a dose of 0.2 ml sometimes caused a significant suppression.

Table 1. Inhibition of the post-castration rise of FSH and LH in acutely castrated male rats by the administration of graded doses of seminal plasma

Treatment	No. of rats	FSH (ng/ml)	LH (ng/ml)
(a) Intact bulls			
0 (saline controls)	7	1161 (1102–1225)	135 (124–150)
0.1 ml	4	1435 (1287–1600)	63 (49–83)**
0.2 ml	4	843 (673–1056)	41 (29–58)**
0.5 ml	4	396 (368–425)**	<20**
(b) Vasectomized bulls			
0 (saline controls)	5	1119 (1029–1219)	479 (367–624)
0.1 ml	4	840 (738–955)*	298 (261–340)
0.2 ml	4	563 (504–630)**	173 (162–184)*
0.5 ml	4	421 (374–474)**	64 (47–99)**

Values are the geometric mean and its 66% confidence interval.

Significantly different from control group; * $P < 0.05$, ** $P < 0.005$.

Table 2. Effect of heat and pepsin treatment on the FSH- and LH-inhibiting activity of Extracts I and V

Treatment	No. of rats	FSH (ng/ml)	LH (ng/ml)
(a) Extract I			
0 (saline control)	8	1205 (1128–1286)	220 (191–252)
40 mg	5	579 (478–700)**	40 (26–63)**
Heat-treated	7	1173 (1063–1295)	144 (111–187)
Pepsin-treated	7	1115 (996–1249)	125 (86–182)
(b) Extract V			
0 (saline control)	9	1580 (1510–1652)	221 (193–254)
23 mg	9	661 (566–772)**	69 (50–95)**
Heat-treated	10	1484 (1382–1593)	315 (292–341)
Pepsin-treated	9	1555 (1403–1725)	231 (185–288)
(c) Extract I			
0 (saline control)	9	1580 (1510–1652)	221 (193–254)
40 mg	8	669 (578–775)**	50 (41–61)**
Incubation at 37°C pH 2, for 24 h	8	754 (648–876)**	76 (54–106)**

Values are the geometric mean and its 66% confidence interval.

Significantly different from control group; ** $P < 0.005$.

The FSH- and LH-inhibiting activity was extracted from seminal plasma by adding cold ethanol to precipitate protein. The activity of Extracts I and V was lost after heating at 67°C for 1 h and after incubation with pepsin (Table 2a and 2b). The loss of activity after pepsin digestion was not due to prolonged heating at 37°C. The activity was retained when Extract I was subjected to the conditions of pepsin incubation, but in the absence of pepsin itself (Table 2c).

Discussion

These results demonstrate that seminal plasma from intact and vasectomized bulls is able to inhibit the post-castration rise of FSH and LH in acutely castrated male rats. This gonadotrophin-inhibiting activity has some properties in common with the FSH-suppressing activity in

bull seminal plasma described by Franchimont *et al.* (1975a) and recently purified and called "bSP-inhibin" by Chari, Duraiswami & Franchimont (1978). The activity in seminal plasma from intact bulls can be precipitated by ethanol, is destroyed by pepsin digestion and preliminary studies show that its chromatographic behaviour on Sephadex G-75 gels eluted with 0.05 M-sodium acetate buffer, pH 4, and with 4 M-urea-0.05 M-sodium acetate buffer, pH 4, is also similar (J. C. Peek & W. B. Watkins, unpublished observations). However, the gonadotrophin-inhibiting activity we have observed in bull seminal plasma has some properties different from those attributed to inhibin. Foremost is its property of suppressing both FSH and LH.

The degree to which material considered to contain inhibin exhibits FSH suppression without accompanying LH suppression varies. For instance, Nandini *et al.* (1976) found that extracts of ram testes suppressed only FSH when given within 12–24 h of castration to 35-day-old rats, while Davies *et al.* (1976), also using acutely castrated 35-day-old rats but a different treatment regimen, reported that extracts from ram rete testis fluid suppressed only FSH at lower doses, but FSH and LH at higher doses. Baker *et al.* (1976) have found that fractions of bull testis homogenates that suppressed FSH alone in sheep suppressed both FSH and LH in intact rats. Such variation may be due to differences in the methods used to detect FSH and LH suppression, or in the material under study. Chari *et al.* (1978) have shown that intravenous injection of freshly prepared "bSP-inhibin" into acutely castrated 35-day-old rats caused suppression of FSH alone, while material which had been stored at -20°C for 6 weeks suppressed both FSH and LH. The seminal plasma samples used in the present study were stored at -20°C for more than 6 weeks before they were used.

The failure of seminal plasma from intact bulls to suppress FSH or LH in chronically castrated male rats, the assay originally used by Franchimont *et al.* (1975a), might be explained by a change in the nature of the response with time after castration (Davies *et al.*, 1976; Hopkinson, Fritze, Chari, Sturm & Hirschhauser, 1977). As yet, we cannot be certain that the gonadotrophin-inhibiting activity observed here is the same as the "bSP-inhibin" described and isolated by Chari *et al.* (1978).

Since the gonadotrophin-inhibiting activities in seminal plasma from intact and vasectomized bulls have the same properties, so far as they have been studied, and are present in the same amount, we suggest that they are due to the same protein material and have the same origin. The activity in the seminal plasma of the vasectomized bulls cannot have arrived from the testis by way of the vas deferens, and so cannot be due to inhibin which has escaped absorption from the rete testis fluid at the epididymis.

Although we cannot exclude the possibility that some inhibin may be present in bull seminal plasma, it seems certain that bull seminal plasma does contain gonadotrophin-inhibiting activity which is not, at least directly, of testicular origin.

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