In Vitro Effect of Inhibin on Cyclic AMP-Phosphodiesterase Activity in Rat Testes

ANIL R. SHETH, S. VIJAYALAKSHMI, PARUL R. SHETH, A. H. BANDIVDEKAR, AND SUDHIR B. MOODBIDRI

From the Institute for Research in Reproduction (ICMR), Parel, Bombay, India

We have previously reported that incubation of testicular slices with inhibin in the presence of 6×10^{-8} M oFSH resulted in a dose-related reduction in accumulation of cyclic AMP due to a decrease in adenyl cyclase activity. The present study demonstrates that inhibin enhances the cyclic AMP-phosphodiesterase activity in rat testicular tissue. These results suggest an additional mechanism by which cyclic AMP levels in gonadal tissue could be regulated by inhibin.

Key words: inhibin, cyclic AMP-phosphodiesterase.

Kraiem and Lunenfeld (1979) reported that human follicular fluid contains an inhibitor of cyclic AMP (cAMP) accumulation. The reduction in cAMP levels could have been due either to decreased production of cAMP (adenyl cyclase inhibition) or to enhanced degradation of cAMP by phosphodiesterase (cAMP-PDE). These authors speculated that the inhibitor of cAMP accumulation may be identical or related to the inhibitors of oocyte maturation and luteinization and may also represent the female analogue of inhibin in the male. We have recently isolated a low molecular weight (1500 daltons) inhibin from ovine testes as well as ovaries (Vijayalakshmi et al, 1980a). These two preparations of inhibin showed identical physico-chemical properties and biological responses, inhibited binding of ^{125}I-hFSH to rat testicular receptors, and also decreased cAMP accumulation (Vijayalakshmi et al, 1980b). In the present investigation, we have examined whether the reduction in cAMP which was observed following inhibin treatment was mediated through the effects of inhibin on phosphodiesterase activity.

Reprint requests: Institute for Research in Reproduction (ICMR), Jehangir Merwanji Street, Parel, Bombay 400 012, India.

Submitted for publication July 14, 1980; revised version received March 18, 1981; accepted for publication May 1, 1981.

Materials and Methods

The inhibin preparation used in the present study was isolated from the high-speed supernatant derived from 40% homogenate of sheep testicular tissue, fractionated sequentially on Sephadex G-100 and Sephadex G-25 columns as described earlier (Sheth et al, 1979; Vijayalakshmi et al, 1980a). The purified preparation of testicular inhibin was used in the present study.

Testes were removed from immature (20- to 22-day-old) and adult (90- to 92-day-old) Holtzman strain male rats. The animals had been housed with free access to water and food in a light-controlled (14 hours of light:10 hours of dark) and temperature-controlled (26 + 1 C) room.

Assay of cAMP-PDE

Slices of decapsulated rat testes were individually pre-incubated for 10 minutes at 37 C under O_2:CO_2 (95:5) in flasks containing 2 ml Krebs Ringer bicarbonate buffer, *p*H 7.0, containing glucose (1 mg/ml). At the end of the pre-incubation period, the buffer was decanted and replaced with fresh buffer containing FSH (6×10^{-8} M oFSH) and different doses of inhibin (25–200 μg). Control flasks contained 200 μg of bacitracin (MW 1500 daltons) or bovine serum albumin. The incubation was continued for an additional hour. At the end of the incubation period, a 20% homogenate of the tissues was prepared in 40 mM tris-EDTA-HCl buffer. The cAMP-PDE activity in the homogenate was determined by measuring the amount of hydrolyzed cAMP using (8-^{3}H) cAMP (20–30 Ci/mmol) purchased from the Radiochemical Centre, Amersham (Thompson et al, 1974). The amount of cold cAMP used was 1 μmol. Results were expressed as μmol cAMP hydrolyzed/min/mg

0196-3635/82/0100/0069/$00.65

TABLE 1. *In Vitro* Effect of Inhibin on the Basal and FSH Stimulated cAMP PDE Activity in Immature Rat Testes*

Basal			In Presence of FSH		
Control		13.5 ± 0.2 (3)	Control		13.5 ± 0.2 (3)
BSA	200/ μg	13.36 ± 0.38 (4)	FSH (6×10^{-8} M)		7.74 ± 0.46§ (4)
Bacitracin	200/ μg	13.82 ± 0.32 (4)	FSH + bacitracin		8.94 ± 0.72 (4)
Inhibin	25/ μg	15.3 ± 0.6‡ (5)	FSH + inhibin	25/ μg	10.12 ± 0.56§ (4)
Inhibin	50/ μg	17.8 ± 0.7§ (4)	FSH + inhibin	50/ μg	12.34 ± 0.56§ (4)
Inhibin	100/ μg	19.9 ± 0.4§ (3)	FSH + inhibin	100/ μg	16.08 ± 0.26§ (4)
Inhibin	200/ μg	27.9 ± 4.7† (3)	FSH + inhibin	200/ μg	19.13 ± 0.24§ (4)

* Values are expressed as mean ± SD/ μM cyclic AMP hydrolysed/min/mg protein; numbers in the parentheses indicate the number of observations.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

protein. Protein estimation was carried out according to the method of Lowry et al (1951) using bovine serum albumin as standard. The significance of treatment effect was assessed by Student's *t* test.

Results

When testes of immature rats were incubated with inhibin at different dose levels (25–200 μg), a dose-related increase in cAMP-PDE activity was observed both in the absence and in the presence of FSH, as seen in Table 1. In testes of adult rats, a significant increase in cAMP-PDE activity was also observed both in the absence and in the presence of FSH; however, this reponse was not dose-related (Table 2). Nonspecific proteins, such as bacitracin and bovine serum albumin, at a concentration of 200 μg, had no effect on cAMP-PDE activity.

Discussion

It is now apparent that inhibin and other low molecular weight peptides (LHRH, TRH, somatostatin) can have multiple sources in the body, can bear multiple physiologic messages for the coordination of different cellular and tissue activities and mechanisms, can have diverse cellular targets, and can have several types of control mechanisms. Inhibin, a specific factor involved in the control of FSH secretion, has been reported to occur in the testes (Moodbidri et al, 1976; Sheth et al, 1979), ovaries (Vijayalakshmi et al, 1980a), and prostate (Vaze et al, 1980). Results of several studies indicate that inhibin could act directly at the hypothalamic (Lugaro et al, 1974; Le Lannou and Chambon, 1977), pituitary (Baker et al, 1976; Rush and Lipner, 1979; Franchimont et al, 1978), and gonadal levels (Moodbidri et al, 1980; Vijayalakshmi et al, 1980b). Evidence collected so far has indicated that inhibin could interfere with the action of FSH by decreasing FSH binding to testicular receptors. This effect of inhibin could lead to decreased adenylate cyclase activity, as we reported earlier (Vijayalakshmi et al, 1980b). Present studies indicate that PDE activity of rat testicular tissue was reduced in the presence of FSH and that

TABLE 2. *In Vitro* Effect of Inhibin on the Basal and FSH Stimulated cAMP PDE Activity in Adult Rat Testes*

Basal			In Presence of FSH		
Control		1.82 ± 0.078 (4)	Control		1.82 ± 0.078 (4)
BSA	200/ μg	1.63 ± 0.2 (4)	FSH (6×10^{-8} M)		1.25 ± 0.10 (4)
Bacitracin	200/ μg	1.7 ± 0.11 (4)	FSH + bacitracin	200/ μg	1.5 ± 0.31 (3)
Inhibin	25/ μg	2.42 ± 0.29‡ (6)	FSH + inhibin	25/ μg	1.81 ± 0.4† (4)
Inhibin	50/ μg	2.35 ± 0.25‡ (6)	FSH + inhibin	50/ μg	2.26 ± 0.24§ (4)
Inhibin	100/ μg	2.57 ± 0.47† (5)	FSH + inhibin	100/ μg	2.44 ± 0.44‡ (4)
Inhibin	200/ μg	2.4 ± 0.57 (6)	FSH + inhibin	200/ μg	2.2 ± 0.56† (4)

* Values are expressed as mean ± SD/ μM cyclic AMP hydrolyzed/min/mg protein; numbers in the parentheses indicate the number of observations.
† $P < 0.05$.
† $P < 0.01$.
§ $P < 0.001$.

inhibin significantly enhanced cAMP-PDE activity. However, this effect of inhibin was dose-related only in immature rat testicular tissue. A dose-dependent decrease in PDE activity with various concentrations of FSH (10–500 ng/ml) in immature rat testes had been reported previously (Means et al, 1976). In contrast, PDE activity in adult rat testes was not demonstrably affected by FSH over the entire range of concentrations tested. Calcium stimulates PDE activity in immature testes, whereas a much less marked effect is noted in the mature animals (Fakunding et al, 1976). Changes in the calcium concentration within the Sertoli cell would alter the activity of cAMP-PDE in immature animals, whereas a similar change in the adult would result in no net difference in cAMP-PDE activity. Indeed, very preliminary evidence suggests that FSH does alter calcium flux in the Sertoli cell of the immature testis (Means et al, 1980).

The present investigation has demonstrated that inhibin enhances cAMP-PDE activity in testicular tissue from immature and adult rats. These results suggest an additional mechanism by which cAMP levels could be regulated by inhibin in gonadal tissue. In view of the well-established role of cAMP in cellular metabolic activity, the action of inhibin on cAMP levels assumes importance in influencing gonadal function.

Deprivation of endogenous inhibin by specific antibodies to inhibin in immature rats resulted in enhanced release of FSH (Sheth et al, in press). Peripheral inhibin levels are higher in immature than in adult rats (Sheth et al, 1978). Inhibin decreased binding of FSH to its receptors to a much greater extent in testes from immature rats than in those from mature animals (Vijayalakshmi et al, 1980b). It appears that inhibin may have a function during gonadal development and differentiation, but once maturity is attained, it may play only a limited role.

References

Baker HWG, Bremner WJ, Burger HG, De Kretser DM, Dulmanis A, Eddie LW, Hudson B, Keogh EJ, Lee VWK, Rennie GG. Testicular control of follicle stimulating hormone secretion. Recent Prog Horm Res 1976; 32:429–465.

Fakunding JL, Tindall DJ, Dedman JR, Mena CR, Means AR. Biochemical actions of follicle stimulating hormone in the Sertoli cell of the rat testis. Endocrinology 1976; 98:392–402.

Franchimont P, De Moulin A, Verstraelen-Proyard J, Hazee-Hagelstein MT, Walton JS, Waites GMH. Nature and mechanisms of action of inhibin: perspective in regulation of male fertility. Int J Androl 1978; 80 (Suppl 2):69–80.

Kraiem Z, Lunenfeld B. cAMP accumulation inhibitor in follicular fluid of human origin. In: Sadler WA. Ovarian follicular development and function. Midgley AR, Jr., New York: Raven Press, 1979; 333–337.

Le Lannou D, Chambon Y. Presence dans l'epididyme d'un facteur inhibant la synthese hypothalamique de FSH, RH chez le rat. C R Seanc Soc Biol 1977; 171:1064–1067.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193:265–275.

Lugaro G, Casellato MM, Mazzola G, Fachini G, Carrea G. Evidence for the existence in spermatozoa of a factor inhibiting the follicle stimulating hormone releasing hormone synthesis. Neuroendocrinology 1974; 15:62–68.

Means AR, Fakunding JL, Huckins C, Tindall DJ, Vitale R. Follicle stimulating hormone, the Sertoli cell, and spermatogenesis. Rec Prog Horm Res 1976; 32:477–527.

Means AR, Deman JR, Tash JS, Tindall DJ, Van Sickle M, Welsch MJ. Regulation of the testis Sertoli cell by follicle stimulating hormone. Ann Rev Physiol 1980; 42:59–70.

Moodbidri SB, Joshi LR, Sheth AR. Isolation of an inhibin like substance from ram testis. IRCS Med Sci 1976; 4:217.

Moodbidri SB, Joshi LR, Sheth AR. On the mechanism of low molecular weight inhibin from ram testes. Ind J Expt Biol 1980; 18:100–102.

Rush ME, Lipner H. Blockade of gonadotropin releasing hormone induced secretion of pituitary follicle stimulating hormone by inhibin containing preparations. Endocrinology 1979; 105:187–194.

Sheth AR, Joshi LR, Moodbidri SB, Rao SS. Characterization of a gonadal factor involved in the control of FSH secretion. J Reprod Fertil 1979; 26:71–85.

Sheth AR, Vanage GR, Vaze AY, Thakur AN. Negative feedback of secretion of follicle stimulating hormone by the pituitary gland of developing male rats. J Endocr (in press).

Sheth AR, Vaze AY, Thakur AN. Development of RIA for inhibin of human seminal plasma and its application for physiological studies. Proceedings of the International Symposium on Recent Advances in Reproduction and Regulation of Fertility, New Delhi, October 1978; 233–240.

Thompson W, Brooker G, Appleman MM. Assay of cyclic nucleotide phosphodiesterases with radioactive substrates. Methods Enzymol 1974; 38:205–212.

Vaze AY, Thakur AN, Sheth AR. Levels of inhibin in human semen and accessory reproductive organs. Andrologia 1980; 12:66.

Vijayalakshmi S, Bandivdekar AH, Joshi LR, Moodbidri SB, Sheth AR. Isolation and characterization of ovine testicular and ovarian inhibin. Arch Androl 1980a; 5:179–188.

Vijayalakshmi S, Moodbidri SB, Bandivdekar AH, Sheth AR. Modulation of FSH action by inhibin. Arch Androl 1980b; 5:213–236.