PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 85–89 (1979)

Effect of Bovine Testicular Extracts on Plasma Gonadotrophins of X-Irradiated Rats (40623)[1]

M. E. RUSH[2] AND H. LIPNER[3]

Endocrine Research Laboratory, Department of Biological Science, Florida State University, Tallahassee, Florida 32306

Swerdloff *et al.* (1) provided indirect evidence that the germinal epithelium of the testis was important in the negative feedback control of FSH secretion in the rat. More recently, other investigators (2–5) have directly demonstrated the presence of a water-soluble testicular factor which prevents the postcastration rise of FSH in the male. This factor was named inhibin by McCullagh (6). Selective destruction of the germinal epithelium by X rays (7), experimental cryptorchidism (1), drugs (8), or ligation of the ductus efferentes (9) induced an increase of blood levels of FSH, but not of LH. We have employed the X-irradiated rat model, referred to as Sertoli Cell Enriched or SCE (10), to demonstrate that an inhibitory factor (inhibin) contained in crude bovine testicular extracts (bTE) can suppress the elevation of FSH and to demonstrate the probable cell of origin of inhibin.

Materials and Methods. Bovine testicular extracts (bTE) were prepared as previously described (2), and bovine kidney extracts (bKE) were similar prepared and used as nonspecific tissue controls. The protein concentrations in the preparations were determined by the Folin–Lowry porcedure (11) and were between 950 and 980 mg/g of lyophilized testicular extract.

To determine the effects of tissue extracts on plasma gonadotrophins, the 34-day-old orchidectomized rat model (2) was used. Immature male rats (34 days) were castrated at 2000 hr. At 0800 hr of Day 35 a blood sample was drawn by a cardiac puncture, and the animal was injected s.c. with vehicle or tissue extract in vehicle. At 2000 hr on Day 35 (24 hr after castration and 12 hr after injection) the animals were exsanguinated, and the plasma was stored at $-20°$ for subsequent gonadotrophin determination.

Male, Sprague–Dawley rats were maintained on a controlled light schedule (12 L:12 D) with free access to food and water in a temperature-controlled room. Thirty-four-day-old rats, whose upper bodies and heads were shielded by lead, received a dose of 500 rads of X rays from a General Electric Maxitron 300 (Tungsten Filament). Nonirradiated littermates were used as controls. Forty-nine days later (postnatal Day 83), blood samples were collected by cardiac puncture under light ether anesthesia. All rats were injected s.c. once daily for 3 days with either 4% gelatin or 250 mg of bTE in 4% gel. Twenty-four hours after the last injection, all rats were castrated and treatments were continued for 3 more days, and on the 4th day after castration, all rats were exsanguinated and the plasmas were frozen for subsequent hormone analysis. The testes, ventral prostates, and seminal vesicles were excised and weighed, and the right testis of each rat was fixed for histological examination.

Plasma concentrations of FSH and LH were measured with the rat radioimmunoassay (RIA) kits generously provided by the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). The intraassay variability for each assay was less than 10%, and, since all bloods were run in the same assay, there was no interassay variability. The assays were run at 37° for 36 hr as described by Moudgal and Madhwa Raj (12). The highly purified NIAMDD-LH and NIAMDD-FSH were labeled with ^{131}I by the

[1] This study was presented in part at the 9th Annual Meeting of the Society for the Study of Reproduction in Philadelphia in 1976.

[2] Present address: Department of Anatomy, University of Nebraska Medical Center, 42nd and Dewey Ave., Omaha, Neb. 68105.

[3] All correspondence should be addressed to: Harry Lipner, Endocrine Research Laboratory, Department of Biological Science, Florida State University, Tallahassee, Fla. 32306.

0037-9727/79/090085-05$01.00/0
Copyright © 1979 by the Society for Experimental Biology and Medicine
All rights reserved.

Chloramine-T method of Greenwood *et al.* (13).

All data were statistically evaluated by a one-way analysis of variance (ANOVA).

Results. In a preliminary study, the biological activity of bTE was demonstrated in 34-day-old, orchidectomized rats (2) with doses ranging from 50 to 250 mg. The postcastration rise of FSH was completely suppressed and that of LH was only partially suppressed (Table I). There was a significant increase of plasma FSH ($P < 0.01$) 12 h after castration (gp 2) as compared to the intact controls. Twelve hours after injection of gel (24 hr after castration) into the castrate controls (gp 2), there was a 47% increase ($P < 0.01$) in plasma FSH levels and a 140% increase ($P < 0.001$) in plasma LH levels. Group 3 rats were administered 100 mg of bTE per rat. These animals exhibited essentially unchanged (−3%) plasma FSH levels (12 vs 24 hr) and a 34% increase ($P < 0.001$) in plasma LH levels. Thus, bTE totally prevented further postcastration rise of plasma FSH and partially prevented the rise of LH. FSH and LH levels in group 4 rats which received 100 mg of bKE did not differ from the castrate control group.

The selective destruction of the germinal epithelium reduced rat testicular weights to approximately 33% of intact control rat testicular weights (Table II). There were no significant differences in the ventral prostate weights among the groups (Table II), although the slight decrease in weights of the SCE groups may be due to some radiation damage to the interstitial cells. There were also no differences in seminal vesicle weights, which were omitted from the data since they did not contribute additional, relevant data.

In addition to the X-irradiation-induced destruction of the germinal epithelium there was a more than two-fold increase of plasma FSH over the intact controls (gps 1 and 2, Table II). In the X-irradiated rats given bTE (gp 3), plasma FSH was suppressed ($P < 0.025$) to the level of intact controls. Although there were no changes in plasma LH levels over the initial treatment period, there was an increase ($P < 0.05$) in LH due to X irradiation. Both gonadotrophins rose in all rats as a result of castration on Day 4. However, continued bTE treatment for 3 days resulted in a 67% suppression of plasma FSH and a 33% suppression of LH as compared to castrate controls (gp 2 vs gp 3, Table II). In a

TABLE I. EFFECTS OF BOVINE TESTICULAR EXTRACTS AND BOVINE KIDNEY EXTRACTS ON PLASMA FSH AND LH IN 34-DAY-OLD CASTRATE MALE RATS[a]

Group	Treatment (No. of animals)	FSH (ng/ml)[b]		LH (ng/ml)[b]	
		12 hr	24 hr	12 hr	24 hr
1	Intact (4)	383 ± 20*	439 ± 21*	49 ± 7*	66 ± 21*
2	Castrate (6)	525 ± 68*	771 ± 71*	148 ± 57*, *	358 ± 37**
3	Castrate + bTE (6)	582 ± 17*	573 ± 18*	113 ± 3*, **	167 ± 5*, **
4	Castrate + bKE[c] (7)	531 ± 22*	675 ± 37*	161 ± 23*, **	428 ± 72**

[a] Injected (s.c.) 8% gelatin, 100 mg bTE in 8% gel, or 100 mg bKE in 8% gel at 12 hr postcastration. All rats exsanguinated 12 hr after the injections (= 24 hr postcastration).

[b] $\bar{X} \pm$ SEM.

[c] bKE was prepared similarly to bTE.

* $P < 0.01$.

** $P < 0.001$.

TABLE II. EFFECT OF BOVINE TESTICULAR EXTRACTS ON X-IRRADIATED RATS[a]

Group	Treatment (no. of rats)[b]	Testes weight (mg)[c]	Ventral prostate weight	FSH (ng/ml)[c]			LH (ng/ml)[c]		
				0 hr	Day 4	Day 8	0 hr	Day 4	Day 8
1	Intact control (6)	3695 ± 67[a]	429 ± 56	383 ± 30[e]	270 ± 18[e]	1942 ± 261[h]	51 ± 8[k]	49 ± 4[k]	453 ± 49[m]
2	SCE (7)	1188 ± 64[b]	427 ± 27	873 ± 54[g]	879 ± 32[g]	2240 ± 324[h]	93 ± 7[l]	100 ± 11[l]	504 ± 75[m]
3	SCE + bTE (9)	1352 ± 64[b]	424 ± 36	819 ± 49[g]	351 ± 30[e]	1283 ± 135[i]	92 ± 14[l]	94 ± 6[l]	285 ± 35[n]
4	SCE + bKE (7)	1324 ± 108[b]	408 ± 27	884 ± 75[g]	968 ± 90[g]	2392 ± 314[h]	88 ± 18[l]	84 ± 16[l]	472 ± 34[m]

[a] Rats were irradiated on postnatal Day 34 and all injections were begun on postnatal Day 83.

[b] Received 1 ml 4% gelatin or 250 mg bTE/ml 4% gel, or 250 mg bKE/ml 4% gel, s.c., once a day for 3 days, castrated on Day 4, and after another 3 days of once daily injections were exsanguinated on Day 8.

[c] $\bar{X} \pm$ SE. $P < 0.05$ = *k, l*. $P < 0.025$ = *e, g*; *h,i*; *m, n*. $P < 0.005$ = *e, g*; *g, h*; *e, i*; *l, n*. $P < 0.001$ = *a, b*; *e, h*; *k, m*.

preliminary study (unpublished), 3 days of bTE treatment (40 mg) in intact rats selectively decreased plasma FSH levels ($P < 0.05$), and had no effect on plasma LH, testicular weights, or ventral prostate weights.

Light micrographs of testicular sections demonstrated the effects of X irradiation on the germinal epithelium. In the X-irradiated testis, no spermatozoa, spermatids, or spermatocytes were seen. Only stem cells spermatogonia, and Sertoli cells were present. They appeared normal according to cell size, shape, and nuclear structure.

Discussion. There have been a number of demonstrations that selective suppression of FSH can be achieved with testicular extracts (2, 5), seminal plasma (3), and rete testicular fluid (4) in castrate males. In this study, using an animal model with selective destruction of the germinal epithelium by X irradiation, bovine testicular extracts suppressed plasma FSH to nonirradiated levels and had no effect on the X-irradiation-induced elevation of plasma LH ($P < 0.05$). The rise of plasma LH levels suggests that some damage to the interstitial cells had occurred. In response to castration the bTE prevented the rise of plasma FSH and LH to untreated castrate rat plasma levels. The bTE consistently suppressed FSH, and only at very large doses suppressed LH in castrated or X-irradiated, immature rats and in cryptorchid rats (preliminary study). Bovine kidney extracts in this model had no effect on FSH or LH.

The ability of the bTE to selectively suppress plasma FSH may seem to be equivocal in this study. However, both a specific effect on FSH only and effects on FSH and LH have been noted with different testicular extracts and experimental protocols in our laboratory (unpublished observations (14)). We have found (14) that high doses or bTE can suppress LH, although in the present study (Table II) this effect was seen only after treatment with bTE for 8 days and castration. This suppression of LH was also observed when high doses of rete testis fluid were administered to male rats (15). Effects on secretion of both gonadotrophins has been previously noted in the literature ((5), see discussion by Swerdloff and Hudson, p. 472 (16)). This dual suppression may be due to potency of the batch of bTE, dose-administered, species of test animal, and/or experimental model employed (for excellent review, see cf. (17)). Recently (18) two models for the regulation of gonadotrophin secretion have been proposed, both of which implicate inhibin and androgens. In the first, androgens control the secretion of FSH and LH, but inhibin controls about one-third of the feedback control of FSH only. In the second model, both inhibin and androgens control both FSH and LH, with inhibin having a greater effect on FSH and androgens more greatly affecting LH. Our data appear to support the second model. However, until inhibin is completely isolated and purified, its effects remain controversial.

It is probable that one of the cellular elements in the seminiferous tubule is the source of inhibin. There is still a question as to whether the Sertoli cell (19, 20), Sertoli-cell/spermatogonial unit (21), or spermatid (22) is the source of inhibin. Destruction of the germinal epithelium by X irradiation leaves only the Sertoli cell and some spermatogonia. If the Sertoli cell or the Sertoli-cell/spermatogonial unit were the source of inhibin, FSH levels should be unaffected by X rays assuming no irradiation damage based on light microscopy. Clinical studies have demonstrated that FSH levels were increased in azoospermic men with testicular histologies of Sertoli cells only or Sertoli cells and germinal cells up to primary spermatocytes, whereas no relationship with FSH levels could be inferred from oligospermic men (5, 22). Baker *et al.* (5) found no significant relationship between FSH levels and Sertoli cell numbers in analyses of cross sections of seminiferous tubules in both fertile and infertile men. These observations, implying germinal cells (spermatid or spermatozoa) as the source of inhibin, are in contrast with those using an *in vitro* Sertoli cell culture system contaminated with less than 20% germ cells (19). Rich and deKretser (20) studied the effect of hydroxyurea (HU) treatment, a chronic vitamin-A-deficient diet, or fetal irradiation on Day 20 of gestation (all of which damaged the germinal epithelium to varying degrees) on serum FSH and Sertoli cell secretory function. Regardless of the amount of germinal cell damage, all treatments were found to impair Sertoli cell secretory func-

tion, as measured by androgen-binding protein (ABP) levels in the testis. Serum FSH levels were increased in all groups. These findings strongly suggest that the Sertoli cell is the source of inhibin. Furthermore, plasma FSH levels were elevated after X irradiation, yet the Sertoli cells appeared to be normal upon observation with the light microscope. However, ultrastructural examination of Sertoli cells, obtained from patients with germinal aplasia, exhibited morphological abnormalities suggestive of impaired (secretory) function (23). These observations coupled with the earlier finding (10) that Sertoli cells may be the *only* target in the testis for FSH strongly suggest that inhibin is produced by the Sertoli cell. Our study does not provide direct evidence for any specific cell type, but lends further support to the hypothesis that the Sertoli cell is the probable cell of origin of inhibin.

Summary. The effect of bovine testicular extracts (bTE) on plasma FSH levels in both castrate and X-irradiated male rats has been examined. Biological activity was demonstrated in 34-day-old, orchidectomized rats with doses ranging from 50 to 250 mg. Testicular weights of X-irradiated rats 49 days after X irradiation were decreased by approximately 67%, whereas the ventral prostate weights were unaffected. Plasma FSH levels (383 ng/ml) increased significantly as a result of X irradiation (873 ng/ml). Plasma LH (51 ng/ml) also rose as a result of X irradiation (93 ng/ml), suggesting that there was some interstitial cell damage or some Sertoli cell damage. Two hundred and fifty milligrams of bTE administered in 4% gelatin given once daily for 3 days suppressed the elevated plasma FSH, but was without effect on plasma LH. Castration and a further 3 days of treatment resulted in a significant postcastration suppression in plasma FSH (control = 1942 ng/ml; bTE = 1283 ng/ml), and resulted in a suppression of LH as well. This LH response (control = 453 ng/ml; bTE = 285 ng/ml) is probably due to the large dose of bTE employed. A bovine kidney extract (bKE) was totally ineffective in suppressing FSH or LH when given at a dose of 250 mg/rat/day in 4% gelatin. These data lend further support to the existence of inhibin, contained in bTE, and to its role as a regulator of FSH in the male. The probable site of origin of inhibin is the Sertoli cell.

We wish to thank Robert Paige, Steven Ray Coleman, A. Trent Williams, Todd Himelberger, Neil Bercow (NSF Secondary Science Training Program), William Vivian, and Harold Jones III, for their assistance. The testes used in this study were obtained from Sunnyland Foods, Inc., Dothan, Alabama through the kind cooperation of Mr. B. Braswell. Also, we thank Dr. Marc E. Freeman for his assistance and criticism, Ms. Linda Derby for her preparation of the manuscript, and Mrs. Ethel Lipner and Mrs. Sally Chapman for their technical assistance. We gratefully acknowledge research support from the Department of Biological Science, Florida State University and from the Tallahassee, Sopchoppy, and Gulf Coast Marine Biological Association. M. E. R. was the recipient of a Grant-in-Aid of Research from Sigma Xi, the Scientific Research Society of North America.

1. Swerdloff, R. S., Walsh, P. C., Jacobs, H. W., and Odell, W. D., Endocrinology **88**, 120 (1971).
2. Nandini, S. G., Lipner, H., and Moudgal, N. R., Endocrinology **98**, 1460 (1976).
3. Franchimont, P., Chari, S., Hagelstein, M. T., and Duraiswami, S., Nature (London) **257**, 402 (1975).
4. Setchell, B. P., and Jacks, F., J. Endocrinol. **62**, 657 (1974).
5. Baker, H. W. G., Bremner, W. J., Burger, H. G., deKretser, D. M., Dulmanis, A., Eddie, L. W., Hudson, B., Keogh, E. J., Lee, V. W. K., and Renie, G. G., Recent Progr. Horm. Res. **32**, 429 (1976).
6. McCullagh, D. R., Science **76**, 19 (1932).
7. Bain, J., and Keene, J., J. Endocrinol. **66**, 279 (1975).
8. Debeljuk, L., and Mancini, R. E., J. Reprod. Fert. **26**, 247 (1971).
9. Setchell, B. P., Main, S. J., and Davies, R. V., J. Endocrinol. **72**, 13 (1976).
10. Fakunding, J. L., Tindall, D. J., Dedman, J. R., Mena, C. R., and Means, A. R., Endocrinology **98**, 392 (1976).
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).
12. Moudgal, N. R., and Madhwa Raj, H. G., *in* "Methods of Hormone Radioimmunoassay" (H. R. Behrman and B. M. Jaffe, eds.), p. 57. Academic Press, New York (1974).
13. Greenwood, F. C., Hunter, W. M., and Glover, J. S., Biochem. J. **89**, 114 (1963).
14. Rush, M. E., and Lipner, H., Endocrinology, **105**, 187 (1979).
15. Davies, R. V., Main, S. J., Young, M. G. W. L., and Setchell, B. P., J. Endocrinol. **68**, 268 (1976).
16. Franchimont, P., Chari, S., and Demoulin, A., J. Reprod. Fertil. **44**, 335 (1975).
17. DeJong, F. H., Mol. Cell. Endocrinol. **13**, 1 (1979).
18. Main, S. J., Davies, R. V., and Setchell, B. P., J. Endocrinol. **79**, 255 (1978).

19. Steinberger, A., and Steinberger, E., Endocrinology **98,** 918 (1976).
20. Rich, K. A., and deKretser, D. M., Endocrinology **101,** 959 (1977).
21. Van Thiel, D. H., Sherins, R. J., Myers, G. H., Jr., and DeVita, V. T., Jr., J. Clin. Invest. **51,** 1009, (1972).
22. Franchimont, P., Millet, D., Vendrely, E., Letawe, J., Legros, J., and Netter, A., J. Clin. Endocrinol. Metabol. **34,** 1033 (1972).
23. Chemes, H. E., Dym, M., Fawcett, D. W., Javadpour, N., and Sherins, R. J., Biol. Reprod. **17,** 108 (1977).

Received July 24, 1978. P.S.E.B.M. 1979, Vol. 162.