Synergistic Role of Prolactin in Response of Male Rat Sex Accessories to Androgen.* (23050)

MORTON D. CHASE, IRVING I. GESCHWIND AND HOWARD A. BERN
(Introduced by C. H. Li)
Department of Zoology, Cancer Research Genetics Laboratory, and Hormone Research Laboratory, University of California, Berkeley.

Huggins and Russell(1) were the first to point out that there is greater prostatic atrophy following hypophysectomy than following gonadectomy in the dog. Other workers(2,3) have reported that hypophysectomized male rats showed less prostatic response to androgen than did animals that had undergone gonadectomy alone. Moreover, several investigators(2,4-8) have concluded that administration of various pituitary factors enhances the response of the ventral prostate and other sex accessories to androgen (or to interstitial cell-stimulating hormone, ICSH, in the presence of the testis). The conclusions derived from these investigations have often been conflicting, and little control has been exercised over the many variables (purity and dosages of pituitary hormones, age and operative condition of rats). Furthermore, in regard to enhancement of ICSH effects, conclusions obtained in this instance do not indicate whether or not the observed effects on the accessories of the administered pituitary hormone are the result of a direct effect upon the sex accessories (*i.e.*, synergism with androgen) or of an increase in androgen secretion by the testis (*i.e.*, synergism with ICSH) or of both effects.

The object of the current study was to ascertain the effects of lactogenic hormone (prolactin, LTH) on response of sex accessories of hypophysectomized-castrate male rats to administration of testosterone propionate (TP). The dose of TP employed was one which caused doubling of the control ventral prostate weights, a response typical of that which follows ICSH administration(8). The dose of LTH was similar to that used by Segaloff(8) and shown by him to modify the response to ICSH administration. The effect of pituitary growth hormone (somatotropin, STH) was also investigated, but only at dose levels selected to control for a possible contamination by STH in the LTH preparations. The use of immature rats at a time long enough after castration and hypophysectomy to eliminate any effects contributed by residual circulating hormones permitted us to determine stimulation of growth, rather than maintenance of development already attained (7).

Materials and methods. 144 male Sprague-Dawley rats were castrated at 28-30 days of age and hypophysectomized 2 days later. Beginning 11 days after hypophysectomy, single daily hormone injections were given for 5 days. Aqueous solutions of LTH and of STH were administered intraperitoneally; TP in sesame oil was injected subcutaneously. Table I lists the various groups of animals and the amounts of hormones used. Rats of Series I and II were obtained from a different source than those of Series III and IV. The LTH was prepared from ovine pituitaries by previously published methods(9,10) and possessed an estimated potency of 35 I.U./mg; the STH was prepared from beef glands by the method of Li(11). On day following 5th injection, rats were sacrificed by neck fracture; ventral prostate, anterior prostate ("coagulating gland"), and seminal vesicles were removed, freed of the bulk of their secretions, and weighed on a 0-50 mg Roller-Smith balance. The *sella turcica* was inspected for evidence of pituitary fragments. The ventral prostate was bisected: one portion was reweighed and immediately frozen on dry ice for later determination of alkaline phosphatase activity(12); the other portion was fixed in Bouin's fluid, serially sectioned

* Aided by Cancer Research Funds of University of California and by N.I.H. of U.S.P.H.S. We wish to thank Mr. Charles Jordan for technical assistance and Dr. G. K. Hawkins, Schering Corp., Bloomfield, N. J., for supplying testosterone propionate (Oreton).

TABLE I. Effect of Testosterone Propionate (TP), Lactogenic Hormone (LTH) and Growth Hormone (STH) on Sex Accessories of Hypophysectomized, Gonadectomized Male Sprague-Dawley Rats.

Series	Daily dose, μg			No. of rats	Body wt, g		Wt, mg§			Glandular tissue in ventral prostate, %§
	TP	LTH	STH		Initial	Final	Ventral prostate	Anterior prostate	Seminal vesicle	
I	0	0	0	10	77	76	6.7 ± .4	2.1 ± .1	5.9 ± .4	
	0	150	0	7	82	88	7.8 ± .6	2.4 ± .1	7.4 ± .4†	
	0	0	7.5	10	70	69	5.9 ± .3	1.4 ± .1	4.9 ± .3	
II	25	0	0	14	83	84	12.4 ± .5	3.7 ± .3	13.9 ± .9	64.2 ± .7 (8)‖
	25	150	0	12	84	86	16.0 ± .7‡	4.6 ± .6	17.3 ± 1.4*	70.3 ± .8‡ (8)
III	25	0	0	8	71	75	15.4 ± .9	4.5 ± .2	15.7 ± .7	66.3 ± 1.1 (8)
	25	150	0	9	70	74	16.6 ± .7	5.2 ± .3	18.9 ± 1.0†	70.4 ± .8‡ (9)
	25	0	7.5	9	69	75	17.7 ± .7	5.2 ± .3	21.3 ± 1.1‡	67.1 ± .9 (9)
IV	25	0	0	13	79	79	15.5 ± .9	5.5 ± .4	22.3 ± 1.4	50.6 ± 1.0 (13)
	25	150	0	10	82	84	17.1 ± 1.1	7.0 ± .5*	27.1 ± 1.5	55.6 ± 1.1‡ (8)
	25	300	0	11	83	85	18.4 ± 1.1	8.3 ± .9†	29.7 ± 2.1‡	
	25	0	3.8	11	83	87	18.7 ± 1.1*	6.9 ± .4*	25.2 ± 1.3	
	25	0	7.5	10	80	84	17.8 ± 1.0	6.3 ± .6	26.1 ± 1.6	51.6 ± 1.3 (10)
	25	150	7.5	10	84	88	21.0 ± 1.1‡	8.1 ± .5‡	32.5 ± 1.2‡	55.7 ± 1.0‡ (9)

* $.02 < p < .05$. † $.01 < p < .02$. ‡ $p \leq .01$. All "p" values were calculated with respect to the first group in each series.

§ Mean ± S.E. of mean. ‖ No. of ventral prostates examined histometrically.

in paraffin, and stained with hematoxylin and eosin or Mallory-Heidenhain stain. In Series II and III, histometric analysis of sections of the ventral prostate was carried out by means of a technic modified from Eränkö(13). The sections from Series IV were studied histometrically with a synchronous scanning device designed by one of us (M.D.C.). In this apparatus, time is used as a measure of distance as the magnified image moves at constant speed past a crosshair superimposed upon the field. This scanning method allowed a more random and hence more objective selection of sections for measurement than the method employed in the earlier series.

Results. In preliminary studies with hypophysectomized - gonadectomized Long-Evans rats, the addition of 150 μg LTH to daily doses of 25-500 μg TP produced no significant increases in the weights of the sex accessories over those of rats receiving TP alone. However, Sprague-Dawley rats subjected to the same treatment showed significant responses (see Series II), and this strain was chosen for further investigation.

Table I summarizes the results. Neither STH nor LTH alone had any stimulatory effects on the ventral or anterior prostates, but LTH apparently elicited minimal growth on the part of the seminal vesicles. TP alone resulted in at least a doubling of the weight of all structures studied over those of controls injected with sesame oil. LTH administered with TP produced a significant increase in the weight of the ventral prostate in Series II, an increase which was accompanied by a concomitant significant increase in the proportion of glandular tissue in this organ.

Since the LTH preparation employed in Series I and II contained a small amount of STH (as indicated by the tibia assay method (14)), an additional group, injected with 7.5 μg STH daily, was introduced to control for a maximal (5%) possible contamination of the LTH with STH. Administration of LTH or STH in Series III resulted in a significant weight increase only in the seminal vesicles; however, LTH effected an increase in the amount of glandular tissue in the ventral prostate, an increase not seen with STH alone.

In the last series (IV), an LTH preparation was employed at 2 dose levels, which, when assayed for growth hormone activity at a total dose of 4 mg, gave no indication of such activity, indicating that any contamination was less than 0.25%. In addition, 2 dose levels of STH were used. Finally, a group was included receiving TP, LTH and STH together, in order to determine whether the in-

dicated individual synergistic effects were additive. The highly purified LTH preparation caused small increases in ventral prostate weight (also evident in Series III) that were not significantly different at either dose level from those observed in the rats treated with TP alone. Significant weight increases were observed in the anterior prostates of both LTH groups and in the seminal vesicles of the groups receiving the dose of 300 μg daily. STH produced no consistent organ weight increments. When 150 μg LTH and 7.5 μg STH were combined with 25 μg TP, highly significant large weight increases (more than 35% above the TP control group) were observed in the ventral prostate, anterior prostate, and seminal vesicles. Significantly more glandular tissue was seen in the ventral prostate in the presence of LTH than in its absence. STH did not have this latter effect.

LTH was shown to have no effect on alkaline phosphatase activity as determined in ventral prostate samples from TP-treated and LTH + TP-treated rats in Series II and III.

Discussion. The sex accessories of rats of the Long-Evans strain in our experiments and in those of Lostroh and Li(7) proved to be unresponsive to the administration of pituitary hormones, even though this strain appeared as sensitive as the Sprague-Dawley to injected androgen after gonadectomy and hypophysectomy. Hence, the results herein reported must be considered as referring to rats of the Sprague-Dawley strain.

Neither LTH nor STH alone was capable of producing significant increases in ventral prostate weight. In only one series (II) did combined LTH and TP administration result in ventral prostates significantly larger than those produced by TP injection alone, and in this series the mean prostate weight of the TP-injected control group was lower than in the other series, in which the animals were obtained from a different source. Nevertheless, despite the failure of LTH to produce a consistent significant increase in the weight of the ventral prostate, a significant increase in the proportion of glandular tissue in that organ was revealed by histometric analysis. Although the combined administration of STH and TP in Series III produced ventral prostate weights equal to or larger than those seen following treatment with LTH, no concomitant increment in glandular tissue was observed with the combined treatment in this series or in Series IV. Neither pituitary hormone, either alone or in conjunction with TP, produced a significant increase in anterior prostate weight in the earlier series. However, with the highly purified LTH of Series IV, significant augmentation of weight of this organ was observed at both dose levels employed. The response to STH, on the other hand, was not so marked and even less regular in occurrence. The seminal vesicle appeared to be the sex accessory in which increase in weight resulted most consistently from the combined administration of LTH and TP. An effect of LTH on the seminal vesicles of gonadectomized rats has been reported previously by Pasqualini(5). In one experiment when STH was administered with TP, a synergistic response was obtained.

The greatest responses to androgen were manifested by all 3 accessories after simultaneous treatment with STH and LTH. In all cases the difference in organ weights between the triply injected animals and those receiving TP alone was found to be significant at "p" values of less than .001. A significant increase in glandular tissue in the ventral prostate was also seen ($p<.01$).

The results indicate that either LTH or STH, at the dose levels employed, is capable of exercising only a minimal degree of synergism with androgen in stimulating male sex accessory growth. This trend of minimal synergism is evident throughout almost all the data; however, statistical evaluation of the data from any single series only occasionally reveals differences that are significant. However, marked synergism, of definite statistical significance, was obtained after simultaneous treatment with all 3 hormones; thus, it appears that both LTH and STH as entities are needed for the optimum synergistic response. Li(15) has recently described STH as a general metabolic hormone, and indeed the increased responsiveness of sex accessories in the presence of STH, and pos-

sibly of LTH, could be viewed in terms of improvement in the general physiologic state of the organism. It would appear, however, that LTH and STH act by different mechanisms, since the former, but not the latter, consistently produces an increase in the proportion of glandular tissue in the ventral prostate.

Summary and conclusions. Lactogenic hormone (LTH) alone or together with testosterone propionate (TP) produced a significant increment in the weight of the seminal vesicle. When given with TP, LTH produced an increase in the amount of glandular tissue in the ventral prostate. Growth hormone (STH) alone or together with TP did not produce any consistently significant increase in the weight of the accessories. When TP, LTH and STH were administered simultaneously to the same test animals, the weight response of all accessories was significantly greater than that to androgen alone. The data are interpreted as indicating that a synergism among the 3 hormones may operate to stimulate sex accessory growth in the male Sprague-Dawley rat.

1. Huggins, C., and Russell, P. S., *Endocrinology,* 1946, v39, 1.

2. Grayhack, J. T., Bunce, P. L., Dearns, J. W., and Scott, W. W., *Bull. Johns Hopkins Hosp.,* 1955, v96, 154.

3. Van der Laan, W. P., *Recent Progress in Hormone Research,* 1953, v8, p287, Academic Press, New York.

4. Paesi, F. J. A., De Jongh, S. E., and Hoogstra, M. J., *Acta physiol. pharm. Neerl.,* 1956, v4, 445.

5. Pasqualini, R. Q., *Rev. Soc. Argentina Biol.,* 1953, v29, 252.

6. Huggins, C., Parsons, F. M., and Jensen, E. V., *Endocrinology,* 1955, v57, 25.

7. Lostroh, A., and Li, C. H., *Rev. Argent. Endocrinol. y Metab.,* 1956, v2, 213.

8. Segaloff, A., Steelman, S. L., and Flores, A., *Endocrinology,* 1956, v59, 233.

9. Li, C. H., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.,* 1942, v146, 627.

10. Cole, R. D., and Li, C. H., *ibid.,* 1955, v213, 197.

11. Li, C. H., *ibid.,* 1954, v211, 555.

12. Huggins, C., and Talalay, P., *ibid.,* 1945, v159, 399.

13. Eränkö, O., *Quantitative Methods in Histology and Microscopic Histochemistry,* 1955, Karger, Basle, pp63-64.

14. Greenspan, F. S., Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinology,* 1949, v45, 455; Geschwind, I. I., and Li, C. H., in *Hypophyseal Growth Hormone, Nature and Actions,* ed. by R. Smith, O. Gaebler, and C. N. H. Long, 1955, Blakiston, New York, pp. 28-53.

15. Li, C. H., *Science,* 1956, v123, 617.

Received January 8, 1957. P.S.E.B.M., 1957, v94.

Effects of Cortisone and Hydrocortisone on Sodium Excretion in Adrenalectomized Rats. (23051)

C. M. KAGAWA AND C. G. VAN ARMAN

Division of Biological Research, G. D. Searle and Co., Chicago, Ill.

Sodium retention with cortisone and hydrocortisone is readily demonstrated in man (1-4). In adrenalectomized rats, commonly used for steroid studies, retention of sodium has not been clearly seen. Singer and Venning(5) in fact observed sodium loss with cortisone, but were unable to obtain a graded response. Results of Johnson(6) and of Dorfman(7) showed increased excretion of the ion with small doses of hydrocortisone, and no effect with larger doses. In our experience, cortisone has caused a loss of sodium with low doses and retention with high doses. It should be carefully noted that a fixed time-interval of urine collection was used for all the foregoing laboratory studies.

Sodium response in rats was therefore further investigated as a function of time and dose following cortisone and hydrocortisone administration. Information of this kind is useful for careful evaluation of sodium-retaining activity in structurally-related steroids. The present report summarizes our data.

Material and methods. Exp. I. Large