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Role of Lactogen in Prostatic Physiology

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Summary. Lactogen causes binding of testosterone to human prostate and thereby increases steroid enhancement of acid phosphatase activity of the tissue. Diminished response to steroid of tissue treated with lactogen antibody is interpreted as evidence of inactivation of endogenous lactogen associated with the tissue. This is the first time that steroid-lactogen-phosphatase interaction has been demonstrated in vitro.

Key words: Prostate, Prolactin, Acid phosphatase, Testosterone.

Introduction

In studies of the enzymatic components of human prostate glands with different histological features, we earlier found that tissues showing predominantly epithelial hyperplasia had greater concentrations of 4-ene-3-oxosteroid-5α-reductase and 3α-hydroxysteroid dehydrogenase than did normal or carcinomatous glands or glands with stromal (fibromuscular) hyperplasia (4). In addition, microsomes prepared from the adenomatous tissue exhibited the highest affinity for the androgens, dihydrotestosterone, 5α-androstane-3α, 17β-diol and dehydroepiandrosterone and the greatest responsiveness of their ($Na^+ + K^+$)-dependent ATPase to the steroids.

Adopting the hypothesis that the activities of the steroid responsive (ATPase) and steroid-metabolizing (reductase and dehydrogenase) enzymes are determined by the accessible concentration of the steroids, we next examined the effect of an androgen synergist, prolactin (5). The finding of Lawrence and Landau (7) that uptake of infused ^{14}C-testosterone into the prostates of hypophysectomized rats was far less than into intact animals had led them and us to suspect that prolactin and/or growth hormone "plays a permissive role in facilitating the full expression of androgenic stimulation of the male accessory organs of reproduction". We, therefore, repeated their experiment in vitro by incubating slices from 34 different human prostates with a medium containing a trace of ^3H-labelled testosterone with and without 2.5 units/ml bovine growth hormone or sheep prolactin (LTH).

With growth hormone the tissue accumulated no more radioactivity than paired slices incubated without protein hormone (3). However, there was a small but significant 8% greater accumulation in tissues treated with LTH than in untreated slices. Of particular interest was the finding that the five glands of the series which exhibited almost pure fibromuscular hyperplasia bound between 20% and 30% more steroid with LTH than without it while the 17 pure adenomas were unaffected by lactogen (binding = 0 ± 10%) (2). Intermediate 10% to 20% increases in steroid uptake were seen in glands having a mixture of adenomatous and fibromuscular growth. This effect of prolactin has been confirmed by Boyns et al. (2) in cultured rat ventral prostates and by Lloyd, Thomas and Mawhinney (8) in incubated rat prostate slices. We found human placental lactogen (HPL) to have an effect identical to LTH (2).

The present work was undertaken in order to find out if the affinity of the tissue for steroid and, secondarily, the biological effect of the androgen is determined by the level of lactogen associated with its cell surfaces.

Materials and Methods

HPL was obtained from Nutritional Biochemicals Company, Cleveland, Ohio, and from Sigma Chemical Company, St. Louis, Missouri. The ^{125}I-labelled HPL, HPL antiserum and normal rabbit serum were obtained from Collaborative Research, Incorporated, Waltham, Massachusetts. ^{3}H-labelled steroids were purchased from New England Nuclear Corporation, Boston, Mass.

Microsomes were isolated from fresh or freshly thawed benign hypertrophic human prostates obtained at surgery. After homogenization of the tissue in 0.25M sucrose-50mM Tris·HCl, pH 7.4, nuclear and mitochondrial fractions were sedimented by centrifugation for 15 min at 15,000 X g and 30 min at 35,000 X g, respectively. The microsomes were obtained by centrifugation of the 35,000 X g supernate for 60 min at 105,000 X g. The pellet was resuspended in Tris · HCl for immediate use.

To study binding of testosterone and HPL to microsomes, an equal volume of microsomal suspension was dispensed into each of a series of tubes containing 46,000 dpm 1,2-^{3}H-testosterone, 40,000 dpm ^{125}I-labelled HPL and various concentrations of unlabelled HPL in 1 ml Tris · HCl buffer, pH 7.4. Following incubation for 1 hour at 37° in air, the particles were sedimented in the centrifuge and the pellet washed with fresh medium, then dissolved in 1 N NaOH. Aliquots of the alkaline solution were counted in a Beckman LS-100 liquid scintillation spectrometer and a Nuclear Chicago gamma counter. After obtaining the dpm ^{125}I from the gamma counter and the counting efficiency for ^{125}I in the beta counter, the beta count was corrected for its ^{125}I component to give the ^{3}H activity present. From the known specific activities of the two labelled species, the p-moles of each bound to the prostatic particles (about 1 mg protein/vessel) was computed.

To study the synergism of lactogen with androgen on acid phosphatase, minced prostate was thoroughly washed in Ringer phosphate buffer, pH 7.4, solution to deplete as much as possible the extracellular stores of phosphatase in acini and ducts. For Experiment B, the tissue was pretreated for 30 min. in Ringer solution containing the indicated serum, then washed before incubation with steroid. The tissue was dispensed into a series of vessels containing Ringer buffer and 5.5 mM glucose plus hormones arranged as indicated in Table 1 and incubated 60 min at 37°. Tissue was separated from medium by centrifugation, washed twice in Ringer solution to deplete extracellular phosphatase, and then homogenized in fresh Ringer buffer solution. After centrifugation, aliquots of the supernatant extract were assayed for prostatic acid phosphatase (6) and the pellet was digested for measurement of its protein concentration (Biuret) so that phosphatase per unit of tissue protein could be calculated.

Results

To find out if the affinity of the tissue for steroid is determined by the level of lactogen associated with its cell surfaces, washed prostatic microsomes were studied as detailed in Methods. While it was recognized that the tissue was probably not free of either endogenous lactogen or androgen we sought to learn if the uptake of additional (exogenous) lactogen would be accompanied by more uptake of exogenous steroid. Fig. 1 shows that steroid binding did increase as more ^{125}I-HPL became associated with the tissue.

Another way to assess lactogen synergism with androgen is to test interaction of the two hormones to modify the acid phosphatase activity of the prostate tissue. Table 1A shows that slices incubated 1 hour with testosterone, 10-^{7}M, had 18% greater activity than controls and this effect was unaffected by the concurrent presence of 2.5 units/ml HPL. However, when the same dose of HPL was added to tissue in the presence of an ineffective (10^{-9}M) level of the steroid, phosphatase activity was almost equal to that

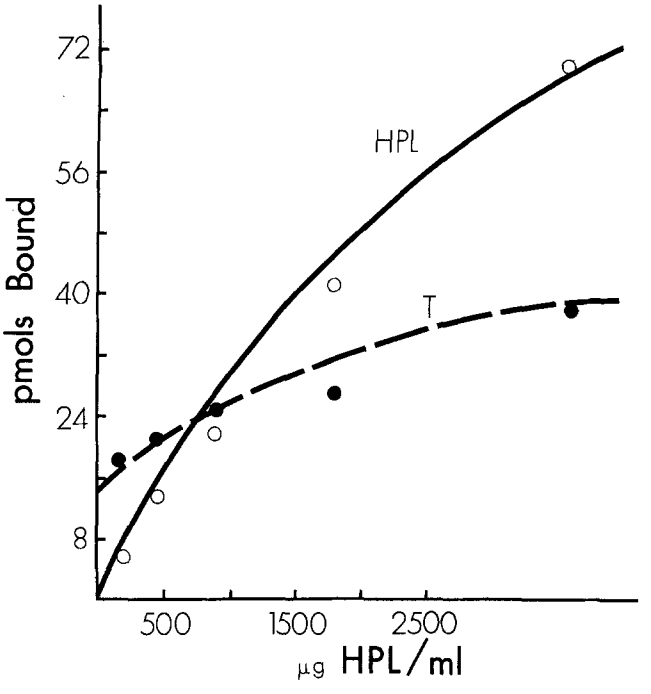

Fig. 1. Influence of lactogen binding on affinity of prostatic microsomes for testosterone. Each point is the mean of 3 trials. See Methods for experimental details

Table 1. Effects of testosterone (T) and lactogen on biosynthesis of prostatic acid phosphatase

Group	Treatment	Trials	Phosphatase activity (% Control ± S.E.M.)
A			
1.	Control	37	100.0 ± 4.7
2.	10^{-7}M T	15	118.2 ± 10.7
3.	10^{-7}M T + 2.5 units/ml HPL	5	120.1 ± 3.1
4.	10^{-9}M T	16	94.1 ± 4.5
5.	10^{-9}M T + 2.5 units/ml HPL	16	115.8 ± 8.5
B			
1.	Control	12	100.0 ± 4.5
2.	10^{-9}M T + 1/400 NRS	12	91.0 ± 6.6
3.	10^{-9}M T + 1/400 Anti-HPL	12	82.1 ± 7.7
4.	10^{-9}M T + 1/200 NRS	12	86.7 ± 8.6
5.	10^{-9}M T + 1/200 Anti-HPL	12	68.3 ± 6.8

See methods for experimental details. T = testosterone; HPL = human placental lactogen; NRS = normal rabbit serum; Anti-HPL = rabbit antiserum to HPL

found with the higher level of androgen alone. This experiment is the first in vitro demonstration of the androgen-lactogen synergism heretofore seen only in vivo. However, it does not provide proof for an action of endogenous prolactin. This problem was approached by inactivating the postulated endogenous prolactin with an HPL antiserum which cross reacts to some degree with human prolactin. Table 1B shows that when minces of human prostate were incubated with the relatively ineffective (10^{-9}M) concentration of testosterone, pretreatment with two concentrations of normal rabbit serum only slightly lowered the phosphatase. When the same concentrations of rabbit anti-HPL were used, phosphatase activity was lowered an additional 10% and 18% respectively presumably through depletion of residual prolactin ($0.1 > P > 0.05$). Such results complement the finding of Asano et al (7) that treatment of rabbits with prolactin antiserum produced dose-related prostatic atrophy.

Discussion

These observations are viewed as tentative demonstration that the amplifying effect of lactogen on prostatic response to androgen is accomplished through increasing the affinity of the gland for steroid. It is suggested that the differences in response to steroid of fibromuscular hyperplastic and adenomatous glands may be due to their relative needs for or content of lactogenic hormone.

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