The Effect of Sex Hormones on the Juxtaglomerular Index of Female Mice

The juxtaglomerular apparatus, a specialized unit composed of portions of both the afferent and efferent glomerular arterioles, the distal convoluted tubule of the glomerulus and extraglomerular mesangial or Lacis cells has been the subject of numerous investigations. The primary function of the apparatus is the production of renin and possibly erythropoietin[1,2]. The juxtaglomerular index (JGI) is a measure of the cytoplasmic granularity of the specialized smooth muscle cells in the afferent arteriole. The JGI is profoundly affected by a large number of conditions including adrenalectomy[3], allograft rejection[4], changes in sodium intake[5], desoxycorticosterone administration[6], glomerular perfusion pressure[7], high altitude[2], magnesium deficiency[8], prolonged spontaneous hypertension[9] and renal ischemia[6]. Despite this comprehensive array of conditions capable of altering the JGI, the effects of sex hormones per se have not been reported. This study was done to determine the effects of long-term administration of progesterone and testosterone on the JGI of mice.

Materials and methods. Six groups of 5-week-old- Swiss mice were used. Males were castrated at the beginning of the experiment with their untreated male littermates used as controls. Progesterone in 0.05 ml sesame oil was administered at the rate of 2 mg per day and testosterone enanthate was adminsitered in 0.05 ml of sesame oil at the rate of 0.2 mg per day. Untreated females and those given daily injections of 0.05 ml sesame oil served as controls. All injections were given subcutaneously. Treatments were continued for 28 days at which time all animals were killed by cervical dislocation and necropsied.

Blocks of kidney, adrenal gland, submaxillary salivary gland, liver and lung were fixed in a 10% solution of neutral buffered formalin and processed for sectioning at 5 μm. All sections were stained with hematoxylin and eosin. For the juxtaglomerular studies, blocks from the kidneys of each animal were sectioned at 2 μm and stained with crystal violet according to HARADA's method[10]. The evaluation of juxtaglomerular granularity in over 6,400 glomeruli was greatly aided by the thin sections and the crystal violet staining technique. All slides were coded prior to examination. The JGI was determined by a modification of an established technique[5]. In addition to the 1+, 2+, 3+, and 4+ grading categories, a 'O' category was added indicating glomeruli with no discernible granularity of the afferent arteriolar cells. Examples of several grades are shown in the Figure. The macula densa was not counted, but isolated arterioles were included when encountered. Two sections from each kidney were systemically scanned with a 10× ocular and 40× objective so that every glomerulus in each section was examined. The 0, 1+, 2+, 3+, and 4+ grading categories were then multiplied by the factors 1, 1, 2, 4, and 8 respectively as described by HARTROFT et al.[5] in order to account for the increased granularity in higher grades. After multiplication, totals of all categories for each section were divided by the total number of glomeruli counted in that section giving the average JGI per glomerulus.

Statistical evaluation of groups was done by ranking individual JGI in the nonparametric Mann-Whitney U-test[11].

Results. The results of JGI computations for all groups are given in the Table. The most striking results were those obtained in the testosterone-treated animals. The JGI was significantly lower than that in untreated control males ($p < 0.01$), untreated control females ($p < 0.025$) and sesame oil treated female controls ($p < 0.01$). Conversely, progesterone administration failed to significantly alter the JGI from that seen in the 2 female control groups. It is interesting to note that untreated male, untreated female, and male castrates all had very similar JGI.

As expected, the sexual dimorphism of the mice was altered by the various treatments. Adrenal 'X' zones were absent in untreated males and testosterone treated females, but were unremarkable or in early degenerative stages in

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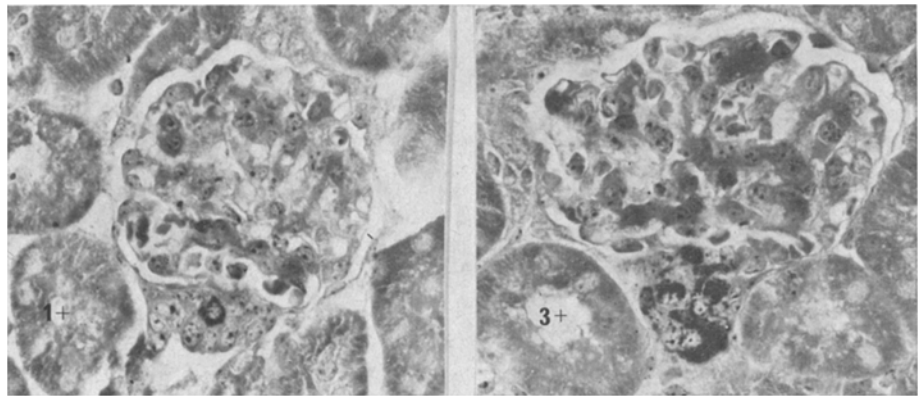

Several examples of empirical grades of granularity assigned to juxtaglomerular cells. The glomerulus on the left has a 1+ designation. Only one cell has granules, which are clustered around the nucleus. The glomerulus on the right is a 3+. Note that many cells have granules dispersed throughout their cytoplasm. Crystal violet, × 600.

Mean juxtaglomerular indices of kidneys from various experimental and control groups

No. of animals	No. of kidneys evaluated	Sex	Treatment	Mean JGI ± S.E.M.
5	9	♂	Castrated	1.60 ± 0.09
5	10	♂	Untreated	1.61 ± 0.07[a]
6	12	♀	2 mg Progesterone daily	1.72 ± 0.08
5	10	♀	0.2 mg Testosterone daily	1.34 ± 0.03
4	8	♀	Untreated	1.64 ± 0.09[b, c]
8	15	♀	0.05 ml sesame oil daily	1.74 ± 0.10[a, c]

[a] Compared to testosterone treated females $p < 0.01$. [b] Compared to testosterone treated females $p < 0.025$. [c] Compared to progesterone treated females $p \geq 0.05$.

all of the other groups. The zona glomerulosa was unremarkable in all groups. Submaxillary salivary glands of untreated males and testosterone treated female animals had convoluted tubular epithelial cells filled with intensely eosinophilic granules characteristic of male morphology. All of the other groups had primarily female morphologies with inconspicuous tubular epithelia and a sparsity of intracytoplasmic granules.

Discussion. Evaluation of the JGI in female mice treated with testosterone has revealed a statistically significant reduction as compared to various control groups. Several possible mechanisms exist by which the JGI could be reduced including a direct response to the androgen. One explanation would be competition of testosterone with aldosterone resulting in decreased salt retention and exhaustion (degranulation) of the juxtaglomerular cells. This explanation, however, seems unlikely since our mice showed no adrenal cortical hyperplasia, a condition which is known to parallel increased aldosterone activity[12, 13]. Testosterone itself causes minor salt retention[14] which could account for the decrease in JGI. The failure of adrenal participation is difficult to assess since it has been shown that JGI changes can be mediated by alterations in cation intake in adrenalectomized animals[15]. In addition, aldosterone is not directly responsible for renin secretion[16]. Another explanation of the decrease in JGI is that the increased blood flow to the kidney as a result of testosterone administration[17] increases glomerular filtration rate and pressure, thereby lowering the JGI[4, 7]. A final possibility could be increased erythropoietin production stimulated by testosterone[18] resulting in decreased JGI.

The inability of progesterone to alter the JGI is difficult to explain[19]. Progesterone administration, unlike testosterone will enhance aldosterone secretion[20] and, therefore should have resulted in a marked decrease in JGI.

In conclusion, the results of this study have suggested that the JGI in Swiss mice is unaffected by sex or orchidectomy and that the JGI is lowered significantly by the chronic administration of high doses of testosterone to female mice[20].

Résumé. La testostérone, administrée de façon chronique à des souris femelles provoque une diminution statistiquement significative de l'index juxtaglomérulaire. Les femelles traitées par la progestérone ou les souris mâles et femelles des groupes contrôlés ne présentent aucune variation. Ces constatations sont discutées en relation avec l'effet de la testostérone sur le flux sanguin rénal, la pression de perfusion glomérulaire et la secrétion d'aldostérone, de rénine et d'érythropoiétine.

E. J. ANDREWS

Department of Comparative Medicine, The Pennsylvania State University, The Milton S. Hershey Medical Center College of Medicine, Hershey (Pennsylvania 17033, USA), 8 January 1972.

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Immunospecificity and Localization of Radiolabeled Human Growth Hormone in the Mouse

Human growth hormone (HGH) has recently been utilized in the clinic for the long term treatment of children with hypopituitary dwarfism[1]. Antibodies to HGH, which are known to contribute to growth inhibition, have been detected in these children[2]. The increased usuage in the clinic and the laboratory synthesis of this protein trophic hormone has prompted investigation into the distribution of radiolabeled HGH in intact experimental animals. In recent studies, the seminal vesicle of the mouse was reported as a target organ for ^{125}I-HGH[3]. The present investigation was undertaken to further clarify the immunospecificity and localization of HGH in the mouse. In addition, studies were undertaken to determine whether HGH radio-uptake occurs in the rat.

Materials and methods HGH, prepared by the RABEN method[4], was supplied and radiolabeled through the courtesy of the Abbott Radiopharmaceutical Laboratories,

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