Renal Retention of Radiothyroxine in Adult Rats Subsequent to Neonatal Gonadectomy and Gonadal Transplanation[1] (34458)

J. W. WAGNER[2] AND W. J. SCHINDLER[3]

Departments of Anatomy and Physiology, Baylor College of Medicine, Houston, Texas 77025

In normal adult female and male rats, the hypothalamus provides, respectively, cyclic or acyclic regulation of gonadotropin secretion. Adult ovarian cyclicity is abolished in female rats exposed to androgen during the first 5 days of life (1, 2). Conversely, the absence of androgen during this early postnatal period in male rats permits differentiation of the hypothalamus along a feminine or cyclic pattern of adult regulation (1, 3, 4). Thus permanent changes in hypothalamic regulation of pituitary gonadotropin function have been produced by neonatal castration or gonadal transplantion (1) or by neonatal androgen administration (2). These neonatal procedures may selectively alter hypothalamic gonadotropin regulatory mechanisms without altering subsequent pituitary–thyroid function or regulation (5, 6).

A rapid and reversible equilibrium exists between thyroxine (T_4) in plasma and tissues, especially the liver and kidneys (7–10). Little is known concerning the factors responsible for T_4 accumulation in these tissues or the significance of this phenomenon. However, a newly emerging concept is that exchangeable intracellular T_4 may be important in the turnover and metabolic action of this hormone (8–10). A marked sex difference in renal retention of T_4 (which appears to be androgen dependent) has been reported in the mouse (11). In the present study, neonatal castration and/or gonadal transplantation was used to alter hypothalamic gonadotropin regulation, and the effect of these procedures on renal retention of T_4 in adult rats was studied as a specific "end organ" which shows a sex difference.

Materials and Methods. Adult female Sprague-Dawley rats originally obtained from Charles River (CD^R) were bred in our colony to obtain the neonatal male and female animals used in these experiments. Following a completely randomized design, newborn female rats were given sc transplants of littermate testes, 1 intact testis to each inguinal region; newborn males were gonadectomized, some of these animals providing donor tissue for the preceding group; newborn males were gonadectomized and given sc inguinal transplants of littermate ovaries; newborn male and female rats were assigned as intact controls. Anesthesia was induced in all animals by hypothermia (5° for 10 min); gonadectomy and/or transplantation was performed (where called for) and, after rewarming under a heat lamp, pups were returned to mothers in litters of 8 animals until weaning at 21 days. All animals were fed Purina lab chow and tap water *ad libitum*. Vaginal smears were taken in all females for several weeks prior to initiation of thyroxine retention studies.

Six rats from each of the five treatment groups were studied at 4–6 months of age. The ^{131}I-labeled thyroxine (sp act 89.5 mCi/mg) contained less than 2% inorganic ^{131}I by paper chromatographic analysis. Carrier thyroxine (T_4) was added, so the solution injected contained 40 μg of L-T_4 and 10 μCi of ^{131}I-labeled L-T_4/ml. The diluent

[1] This investigation was supported in part by USPHS Research Grants HD 2200 and HD 01630 from the National Institute of Child Health and Human Development and FR 05425. Computer time was supported in part by USPHS FR 00254.

[2] Recipient of USPHS Research Career Development Award 1 K3 HD 16415 from the National Institute of Child Health and Human Development.

[3] Recipient of USPHS Research Career Development Award 5 K3 AM 5928 from the National Institute of Arthritis and Metabolic Diseases.

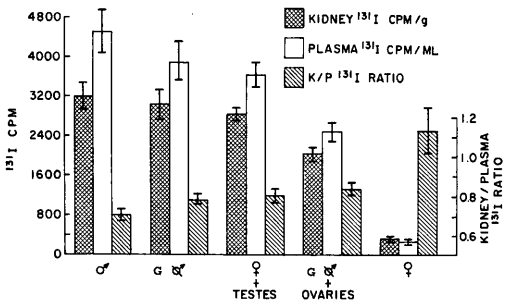

FIG. 1. Renal and plasma radioactivity 24 hr after the last of 7 daily injections of ^{131}I-T_4 plus carrier T_4 in groups of rats 4–6 months of age. The ^{131}I in both plasma and kidneys was about 93% TCA precipitable. Bars represent group mean G♂ SE mean; 6 rats/group; ♂ = intact males; G♂ = gonadectomized males; ♀ + testes = females with sc testicular transplants; G♂ + ovaries = gonadectomized males sc ovarian transplants; ♀ = intact females; all operations and transplants were done on newborn animals.

consisted of 50% propylene glycol, 0.8% methanol: NH_4OH (3:1) and the remainder, of 1% bovine albumin in saline. Each rat was injected sc daily for 7 days with 0.1 ml/100 g of body wt of this solution. Thus, each rat received daily 4 μg of T_4 and 1 μCi of ^{131}I-labeled T_4/100 g of body wt. All animals were killed 24 hr after the final injection. Animals were anesthetized with ether and blood was collected in heparinized centrifuge tubes from incisions in the abdominal aorta and vena cava. After centrifugation, the radioactivity in 0.5 ml of plasma was counted in a well-type scintillation counter. Kidneys were removed, cleaned of connective tissue capsule, weighed, and the radioactivity was similarly counted.

In situ gonads, uteri, adrenals, thyroids, pituitaries, and transplanted gonads were removed, cleaned, and weighed to the nearest 0.05 mg at autopsy; all gonads, both *in situ* and transplants, were fixed, embedded in paraffin, sectioned at 8 μ and stained with hematoxylin and eosin for histological study. Statistical probabilities were determined with analysis of variance when only two groups were compared and with the multiple range test of Duncan (12) when more than two groups were compared. Probabilities greater than 5% were considered nonsignificant. All values reported represent group means ± standard error of the mean.

Results. The renal content of radioactivity 24 hr after the last of 7 daily injections of ^{131}I-labeled T_4 plus carrier T_4 is shown in Fig. 1. 93.4% of the kidney radioactivity was precipitated by 10% trichloroacetic acid. Under similar experimental conditions, Kamei *et al.* (11) demonstrated that renal radioactivity in the mouse was in the form of ^{131}I-labeled T_4. Radioactivity in the kidneys of all groups of rats was significantly higher (6–10 ×) than that in normal females ($p<0.001$). Renal radioactivity (^{131}I, cpm/g of tissue) in normal males, castrated males, and females with testes was significantly higher ($p<0.01$) than in castrated males with ovarian transplants. Plasma radioactivity (92.8% of which was trichloroacetic acid precipitable) paralleled that of renal radioactivity, being highest in normal males and significantly lower in normal females ($p<0.001$) than in all other treatment groups (Fig. 1). The kidney to plasma radioactivity concentration ratio (K/P ratio) was greater than one only in the normal females and was significantly higher in normal females ($p<0.01$) than in all other groups (Fig. 1).

Table I summarizes the effects of the various treatments on body and organ weights. As expected, castrated males (group B) or those castrated and given transplanted ovaries (group D) weighed significantly less than intact males (group A). Androgen secreted during the neonatal period by testicular transplants did not have any significant effect on body weights in adult female rats (compare groups C and E). Relative kidney weights were not affected by neonatal treatments. Adrenals and pituitaries of females with testicular transplants (group C) and gonadectomized males with ovarian transplants (group D) were markedly enlarged on both an absolute and relative weight basis. Vaginal smears from females given testicular transplants at birth (group C) showed persistent vaginal cornification for more than 4 weeks prior to autospy. *In situ* ovaries of these animals were significantly smaller ($p <$ 0.001) than those of normal females and con-

TABLE I. Absolute and Relative Organ Weights and Body Weights of Rats 4–6 Months of Age Subsequent to Neonatal Gonadectomy and/or Gonadal Transplantation.[a]

Group	Body wt (g)	Kidney (g)	Kidney (g/100 g of body wt)	Adrenals (mg)	Adrenals (mg/100 g of body wt)	Ant. pituitary (mg)	Ant. pituitary (mg/100 g of body wt)	Thyroid (mg)	Thyroid (mg/100 g of body wt)	Ovaries (mg)	Ovaries (mg/100 g of body wt)	Uterus (mg)	Uterus (mg/100 g of body wt)
A. Male intact	388 ±21	2.611 ±0.15	0.67 ±0.02	44.9 ±2.3	11.6 ±0.7	7.5 ±0.5	1.9 ±0.08	11.6 ±1.2	3.0 ±0.30				
B. Male neonatal gonadectomized	319 ±19	1.775 ±0.07	0.56 ±0.02	55.7 ±1.6	17.8 ±1.2	9.8 ±1.1	3.1 ±0.39	14.1 ±2.3	4.4 ±0.56				
C. Female neonatal testes transplants	269 ±4	1.845 ±0.06	0.69 ±0.02	68.0 ±5.9	25.1 ±1.9	14.9 ±1.0	5.5 ±0.36	13.3 ±0.6	5.0 ±0.16	36.1 ±2.4	13.4 ±0.86	556.5 ±47.2	206.2 ±15.8
D. Male neonatal gonadectomized + ovarian transplants	308 ±7	1.922 ±0.12	0.62 ±0.03	79.9 ±6.8	25.7 ±2.6	15.5 ±1.7	5.0 ±0.52	10.2 ±1.3	3.3 ±0.44				
E. Female intact	258 ±3	1.480 ±0.06	0.57 ±0.02	53.0 ±2.2	20.5 ±0.9	10.7 ±0.8	4.1 ±0.30	10.9 ±1.1	4.2 ±0.40	60.8 ±2.9	23.5 ±0.88	568.3 ±33.2	220.1 ±12.2
Multiple comparisons[b]	A vs. all** B vs. E* D vs. E*	A vs. all** D vs. E** C vs. E* B vs. E*	NS	D vs. A, E, B** C vs. A** C vs. E, B*	D vs. A, B** D vs. E* C vs. A, B** D vs. E* E vs. A** B vs. A**	D vs. A, B, E** C vs. A, B, E** E vs. A*	C vs. A, B, E** D vs. A, B** E vs. A** E vs. B* B vs. A*	NS	C vs. A** C vs. D* B vs. A* E vs. A*	***	***	NS	NS

[a] All values represent mean ± standard error of the mean; 6 rats/group.

[b] * or ** significantly different (at 5 or 1%, respectively) according to the multiple range test of Duncan when multiple groups compared; analysis of variance when only two groups compared. Only significant differences are noted in multiple comparisons. NS = nonsignificant, $p > 0.05$. *** $p < 0.001$.

tained only small and, occasionally, cystic follicles with no corpora lutea. Serial sections of testicular transplants (group C) showed extensive regression of tubules and complete necrosis of interstitial cells. Histological examination of ovarian grafts from neonatally castrated males (group D) showed extensive corpus luteum development.

Discussion. From vaginal smear, ovarian and organ weight data (*i.e.*, persistent vaginal cornification, small ovaries lacking corpora lutea, and enlargement of adrenals and pituitary), it is apparent that the neonatally transplanted testes were functional during the early differentiation period and thereby produced the classical effects in these animals (group C), namely, an acyclic secretion of gonadotropin and an anovulatory condition. The necrotic state of testicular transplants observed at autopsy in these animals would indicate that the grafts were no longer capable of androgen secretion at the time of these experiments. The finding of corpora lutea in ovarian transplants of neonatally gonadectomized males (group D) suggests that these grafts were functional during the neonatal period and, along with the adrenal and pituitary enlargement seen at autopsy, indicates that these grafts were probably secreting ovarian hormones at the time these experiments were made.

Kamei and co-workers (11) reported that adult male mice retained more labeled T_4 in their kidneys following injection of labeled plus pharmacological doses of T_4 than did adult female mice. As a preliminary to the present study, the original observation of a marked sex difference in renal retention of T_4 in adult mice was confirmed. This sex-dependent distribution of thyroidal hormone was then studied in the rat as one aspect of adult extrathyroidal physiology which might be influenced by neonatal gonadal hormone manipulation. These studies extend the observation of Kamei and co-workers to another species where, again, normal male rats retained almost 10 times as much T_4 in their kidneys as did normal females. The term, renal retention of radiothyroxine, as used herein refers only to the renal radioactivity (cpm/g) 24 hr after the last of 7 daily injections of carrier plus labeled T_4 and does not imply anything concerning the mechanism or significance of kidney accumulation of T_4. More detailed kinetic studies providing some information about these parameters have recently been published (7–9). In the studies reported here [and also those of Kamei *et al.* (11)], T_4 was injected on a body weight basis, thus the males of both species, being heavier than females, received more T_4. To eliminate dose of T_4 as a factor in the increased renal retention of T_4 by males, another experiment was performed on additional groups of adult male and female rats which were all injected with the same dose of ^{131}I-labeled and unlabeled T_4. The results were identical to those reported herein; thus, the injected dose of T_4 did not account for the sex difference in renal retention. Inorganic ^{131}I contamination of the ^{131}I-labeled T_4 used would not account for the sex differences observed in these experiments since the kidney radioiodide concentration (cpm/g) and the *K/P* [iodide] ratio in adult rats of similar treatment groups to those used in these studies were not altered by neonatal procedures. In fact, the kidney concentration of radioiodide was higher in females than males (our unpublished observations).

A species variation between mice and rats with regard to sex difference in kidney/plasma radiothyroxine concentration ratios was observed. In mice, plasma radiothyroxine levels were lower in males than females, resulting in significantly higher kidney/plasma radiothyroxine concentration ratios in males [(11) and our unpublished observations]. Plasma radiothyroxine levels were significantly higher in male rats than females, resulting in significantly lower *K/P* radiothyroxine concentration ratios in males (Fig. 1).

The suggestions of Kamei *et al.* (11) that in the mouse, renal retention of radiothyroxine is related primarily to androgen levels in the adult male or that androgen susceptibility may be sex-linked genetically and thus restricted to the male sex, do not appear as likely explanations for our observations since neonatal castration in male rats did not re-

duce renal retention of radiothyroxine significantly below that of normal males. This may represent another species difference between rats and mice.

Further, neonatal transplantation of testes (which were not secreting androgen at the time of study) into female rats raised the renal retention of radio thyroxine to a level not significantly different from that seen in normal males while neonatally castrated male rats bearing ovarian grafts exhibited a significant reduction in renal retention of radiothyroxine, but not to levels as low as those observed in normal females. From the present studies, it would seem that when a pattern for tonic gonadotropin secretion is established, renal retention of radiothyroxine is elevated, as in the normal male, the female treated neonatally with androgen, or the neonatally castrated male (which, lacking estrogen feedback from transplanted ovaries, does not manifest cyclicity). When the capacity for cyclic gonadotropin release is established, renal T_4 retention is reduced as in the normal female or the neonatally castrated male bearing ovaries.

Under the conditions of these experiments, neonatal manipulations which are known to influence adult patterns of gonadotropin secretion also altered the level of renal retention of radiothyroxine in the direction of the intact, genetically opposite sex. In unpublished work (on groups of rats similar to those in this report) we have observed that the fractional turnover rate (K) of plasma inorganic iodide measured in a cold (5°) environment was almost twice as rapid in normal female rats as in normal males ($K = 11.4 \pm 0.7$ and $6.5 \pm 0.7\%$/hr, respectively). In male experimental animals, neither neonatal gonadectomy nor gonadectomy plus ovarian transplantation altered the slow male-type disappearance rate. Females given testicular transplants as neonates still retained the rapid plasma radioiodide disappearance rate of normal females. It is therefore interesting to note that the neonatal procedures used in these experiments may alter certain aspects of extrathyroidal T_4 metabolism (as reported herein) while leaving extrathyroidal iodide metabolism unchanged.

Although the mechanism(s) underlying this phenomenon of sexual dimorphism in renal retention of radiothyroxine cannot be established from observations in this report, several possibilities are worth consideration. The fractional turnover rate of plasmathyroxine is significantly faster in female rats than in males (6). This may partially account for the sex differences in renal T_4 retention which were observed. The binding of thyroxine to plasma proteins contributes to the regulation of its passage across the cell wall (13). For example, the uptake of radiothyroxine by tissue slices of liver, heart, and kidney cortex *in vitro* was depressed by the addition of thyroxine-binding proteins or serum (containing these proteins) to the medium (14). Hormonal effects on thyroxine binding proteins might secondarily influence T_4 flux into the kidney. In the rat, enterohepatic circulation of T_4 is extremely important, accounting for the major excretory pathway of T_4 via the feces (15). Estrogen has significantly increased the urinary and fecal excretion of radioactivity following administration of labeled T_4 in rats (16). When liver uptake of T_4 is diminished, kidney uptake is increased (17). The above reports suggest that hormonal effects on liver metabolism and/or uptake of T_4 might also indirectly influence its retention by the kidneys. Evaluation of the effects of variations in circulating estrogen and/or progesterone levels, both of which occur in the normal female and neonatally castrated male bearing ovarian transplants and are associated with decreased renal retention, may provide further insight into the basis for the sex difference in this phenomenon.

Summary. Neonatally castrated male rats (some bearing ovarian transplants) and female rats given testicular grafts at birth were injected chronically with ^{131}I-labeled L-T_4 and carrier T_4 as adults; the radioactivity in plasma and kidneys was measured and compared with that in normal adult males and females. Both renal and plasma radioactivity were significantly higher in normal male rats than in females. Neonatally castrated males as well as females given testicular transplants

at birth exhibited an increase in renal T_4 to levels of normal males whereas neonatally castrated males bearing sc ovarian transplants showed a significant reduction in renal retention of T_4. A sex difference in the capacity of the kidney to concentrate radiothyroxine was demonstrated. This was not solely related to adult androgen levels and was influenced by neonatal hormonal manipulations which are known to alter hypothalamic–pituitary relationships in the adult rat.

The skilled technical assistance of Mr. Phil Cooper and Mrs. Joy Ewing is most gratefully acknowledged. Statistical analyses were done with the program EXBIOL on file at the Common Research Computer Facility, Texas Medical Center, Houston, Texas. The authors are indebted to Dr. Edvart Sakiz for this program.

1. Pfeiffer, C. A., Am. J. Anat. **58**, 195 (1936).

2. Barraclough, C. A. and Gorski, R. A., Endocrinology **68**, 68 (1961).

3. Harris, G. W., Endocrinology **75**, 627 (1964).

4. Gorski, R. A. and Wagner, J. W., Endocrinology **76**, 226 (1965).

5. Brown-Grant, K., J. Physiol. **176**, 91 (1964).

6. Schindler, W. J. and Wagner, J. W. Neuroendocrinology (1970) in press.

7. Gorman, C. A., Flock, E. V., Owen, C. A., and Paris, J., Endocrinology **79**, 391 (1966).

8. Hansen, J., Bernstein, G., Volpert, E., and Oppenheimer, J. H., Endocrinology **82**, 37 (1968).

9. Oppenheimer, J. H., Bernstein, G., and Surks, M. I., J. Clin. Invest. **47**, 1399 (1968).

10. Oppenheimer, J. H., New Engl. J. Med. **278**, 1153 (1968).

11. Kamei, T., Tonoue, T., Suzuki, M., and Yamamoto, K., Endocrinology **77**, 879 (1965).

12. Duncan, D. B., Biometrics **11**, 1 (1955).

13. Pitt-Rivers, R. and Tata, J. R., "The Thyroid Hormones." Pergamon, Long Island City, N. Y. (1959).

14. Freinkel, N., Ingbar, S. H., and Dowling, J. T., J. Clin. Invest. **36**, 25 (1957).

15. Gross, J. and LeBlond, C. P., J. Biol. Chem. **171**, 309 (1947).

16. Grosvenor, C. K., Endocrinology **70**, 673 (1962).

17. Oppenheimer, J. H., Bernstein, G., and Hansen, J., J. Clin. Invest. **46**, 762 (1967).

Received Sept. 22, 1969. P.S.E.B.M., 1970, Vol. 133.