Sex Differences in Organic Ion Transport by Rat Kidney[1] (36754)

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The renal handling of certain compounds appears to be sex linked in some species. Harvey and Malvin (1) observed that creatinine clearance was higher in male than female rats. Testosterone induced tubular secretion of creatinine in females (1). Huang and McIntosh (2) and Ferguson (3) demonstrated that renal cortical slices from male rats accumulated more *p*-aminohippurate (PAH) than did slices from females. Inasmuch as both creatinine and PAH are transported as anions it was of interest to determine if the effect of testosterone on anion transport was specific or reflected a more general trophic effect on the kidney. The specific purpose of this study was to determine the specificity of the sex difference in renal transport of organic anions by determining the effect of sex on renal uptake of PAH and the organic base tetraethylammonium (TEA). Furthermore, these experiments were designed to elucidate the mechanism responsible for the difference in PAH uptake between male and female rat renal cortex.

Methods. Sexually mature male and female Sprague-Dawley rats (12 weeks old) were killed by cervical dislocation and their kidneys removed immediately, weighed, and placed in ice cold normal saline. Renal cortical slices were prepared free hand and kept briefly in cold normal saline until incubated.

Approximately 80–100 mg of slices were incubated in 2.7 ml of the phosphate buffer devised by Cross and Taggart (4), which contained 7.4×10^{-5} *M* PAH and 1.0×10^{-5}*M* TEA-^{14}C (1.15 mCi/mmole). All incubations were carried out in a Dubnoff metabolic shaker at 25° under a gas phase of 100% oxygen. Incubation time varied from 30 to 180 min. After incubation the slices were quickly removed from the beakers, blotted and weighed. The weighed slices were macerated in 3 ml of cold trichloroacetic acid (10%). The final volume of the homogenates was brought to 10 ml with distilled water and centrifuged. A 2 ml aliquot of media was similarly treated. PAH in the supernatant was estimated by the method of Smith *et al.* (5), and TEA-^{14}C was counted in a Beckman LS-100 liquid scintillation counter with a modified Bray's solution (6.0 g of 2, 5-diphenyloxazole and 100 g of naphthalene/liter of dioxane). Results were expressed as slice to medium (*S*/*M*) ratios, where *S* equals milligrams per gram of tissue (wet wt) or disintegrations per minute per gram of tissue and *M* equals milligrams per milliliter of medium or disintegrations per minute per milliliter of medium.

Extracellular space of renal cortical slices was measured by the inulin space method of Weber and Cairns (6), using inulin-^{14}C (2 mCi/g). Incubations were carried out for 90 min with beakers containing 50 to 100 mg of tissue and 0.075 μCi of inulin-^{14}C in 3 ml of standard medium. The inulin space as a fraction of wet weight was estimated from the tissue and medium concentration of inulin. Total water content was determined by weighing tissue before and after drying for 24 hr at 100°. Intracellular space was calculated as the difference between total water content and inulin space. Slices were analyzed for protein content by the method of Lowry *et al.* (7).

Runout of *p*-aminohippuric-(glycyl-2-^{3}H) acid (106.6 mCi/mmole) was determined by the method of Farah, Frazier and Stoffel (8). Preliminary loading of the slices with PAH-^{3}H was accomplished by incubating at

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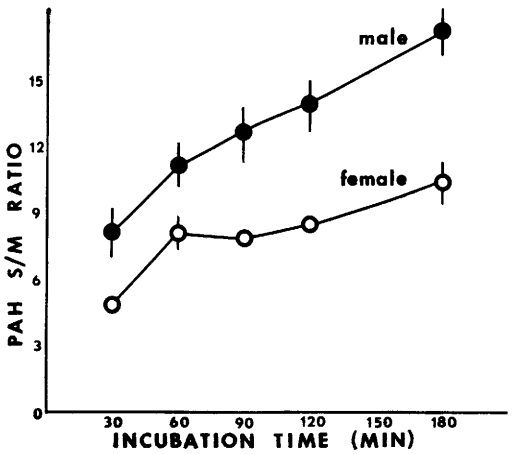

FIG. 1. Accumulation of PAH by renal cortical slices of adult male and female rats. Kidney cortical slices from 3 to 4 animals were pooled and equally divided in a series of beakers, which were incubated for times ranging from 30 to 180 min. Points indicate means (± SE) for six such experiments. Standard error was not shown in those cases where the vertical bars were less than the diameter of the points.

25° for 2 hr in the normal medium containing 6.0 × 10^{-4} *M* PAH-^{3}H. The slices were removed from the PAH-^{3}H containing medium blotted on gauze and transferred at 1 min intervals through a series of 12 beakers containing normal medium, free of PAH. All runout beakers were equilibrated for 30 min at 25° under a gas phase of 100% oxygen prior to runout. Runout experiments were performed in the Dubnoff metabolic shaker at 25° under a gas phase of 100% oxygen. The amount of PAH-^{3}H found in the runout beakers was determined and the runout curve was plotted.

To estimate rate of PAH transport, slices were incubated for 2 and 12 min at varying PAH concentrations (1, 2, 4 and 8 × 10^{-4} *M*). The rate of uptake was calculated as the difference between uptake at the two incubations divided by 10. The results were plotted using a Lineweaver-Burke plot [cited in (9)], where the reciprocal of the rate of PAH uptake per minute was plotted against the reciprocal of PAH concentration.

Data were expressed as means ± SE and were analyzed using Student's *t* test for unpaired observations. The level of significance was chosen as $p < .05$.

Results. Adult male rats are heavier than adult females. Similarly, kidneys from males are heavier than kidneys from females. However, when kidney weight was factored by body weight the ratio for six typical males (0.0067 ± 0.0001) was no different than that of six typical females (0.0066 ± 0.0002). Nevertheless, renal cortical slices from male rats accumulated more PAH than slices from females (Fig. 1). After 90 min of incubation, slices from males developed a PAH *S/M* ratio of 12.7 ± 1.0 while an *S/M* ratio of 7.8 ± 0.3 was seen with tissue from females. This difference was independent of incubation time from 30 to 180 min (Fig. 1).

The TEA *S/M* ratio for renal cortical slices from male rats was significantly greater than from females. The difference in TEA *S/M* ratio (3.1 ± 0.4 at 10^{-5} *M* TEA) between renal cortical slices from male and female rats was magnified to 9.24 ± 1.1 with 10^{-7} *M* TEA in the medium (Fig. 2).

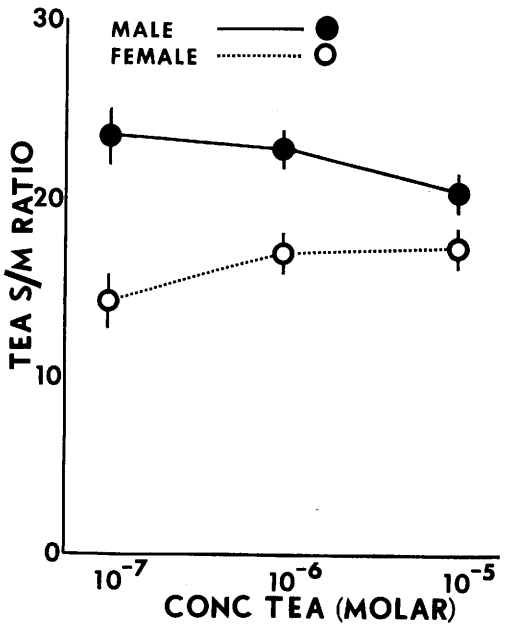

FIG. 2. Effect of increasing medium concentration upon the accumulation (*S/M*) of TEA by male and female rat renal cortical slices. Kidney cortical slices from 3 to 4 animals were pooled, equally divided, and incubated for 90 min at the different concentrations. Points indicate means (± SE) from six experiments.

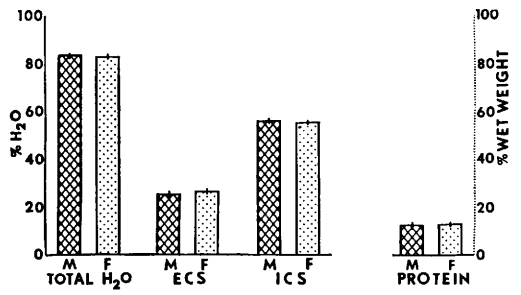

FIG. 3. Comparison of cortical slice composition from male and female rat kidneys. Total water content, extracellular space (ECS), intracellular space (ICS) and protein content of male (M) and female (F) renal cortical slices were measured and expressed as a percentage of tissue wet weight. Bars indicate means (± SE) from six experiments.

Total water content (expressed as percentage of tissue wet wt) of renal cortical slices from male (83.9 ± 0.5%) and female (83.3 ± 0.5%) rats was not different (Fig. 3). Extracellular space (inulin space) was 25.4 ± 0.9% for males and 26.6 ± 0.6% for females. Consequently, intracellular space, the difference between these two measurements, was not different. Similarly, protein content of the cortex from female rats (12.7 ± 0.7%) was no different than that of the males (12.2 ± 0.5%).

Runout of PAH from preloaded renal cortical slices was exponential (Fig. 4). The runout curves for PAH appear to be a composite of at least two separate components. The slow component of the curve could be described by the equation for a first order reaction. The rate constant k for this part of the curve was calculated using the equation for a first order reaction:

$$k = \frac{2.303}{t} \log \times \frac{\text{concn of PAH in the slice at zero time}}{\text{concn of PAH in the slice at time } t}$$

The rate constant describing disappearance of PAH via the slow component from male tissue (0.046 ± 0.004 min^{-1}) was less than that from female tissue (0.070 ± 0.020 min^{-1}). This difference in rate constant for one portion of the curve was accompanied by a longer half-time of runout from the entire curve. The half-time for male tissue was 8.0 ± 0.81 min, compared to 6.0 ± 1.4 min for female tissue.

Rate of PAH uptake was determined at concentrations of 1, 2, 4 and 8 × 10^{-4} M PAH. The rate of PAH uptake for renal cortical slices from males was significantly greater than for slices from females. The experimental results were plotted on a double reciprocal plot and are expressed in Fig. 5. The slopes of lines for male and female were 0.217 and 0.373, respectively. The plot for male tissue exhibited a V_{max} (units of μg/g/min) of 27.02 while the V_{max} for the female was 7.75. Apparent K_m values for male and female tissue were 5.86 and 2.89 × 10^{-4} M, respectively.

Discussion. The PAH S/M ratios developed at 90 min incubation for male (12.7 ± 1.0) and female (7.8 ± 0.3) are comparable to the values (15.4 ± 1.0 and 8.4 ±

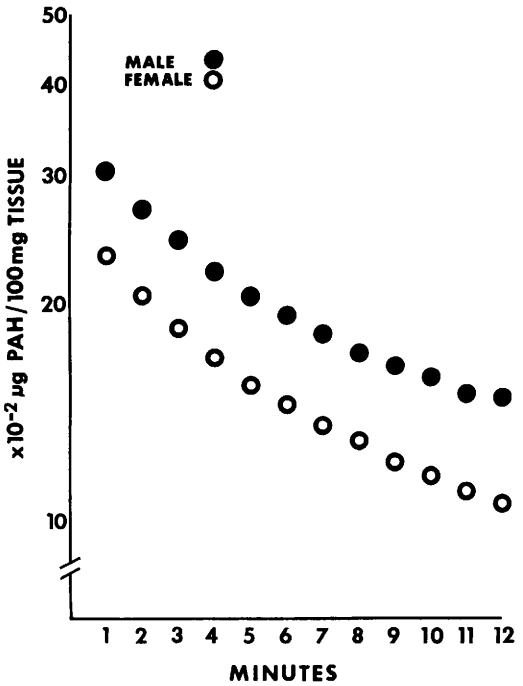

FIG. 4. Runout of PAH from renal cortical slices of adult male and female rats. Slices from 2 animals were pooled and preloaded for 2 hr with PAH and then transferred at 1 min intervals into a series of 12 PAH-free beakers. Data represent concentration of PAH remaining in slices (initial concentration less amount appearing in beakers). Points indicate means for four such experiments.

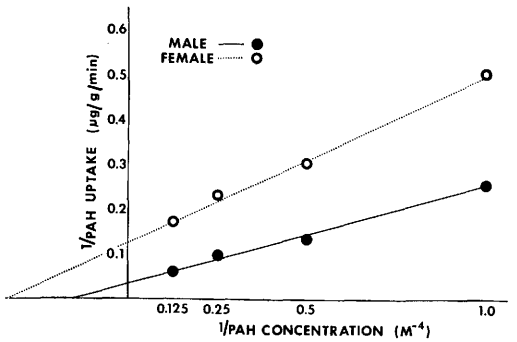

FIG. 5. Kinetic analysis of PAH uptake by renal cortical slices. The rate of PAH uptake μg PAH/g/min) at PAH concentrations of 1, 2, 4 and 8 $\times$ 10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 min of incubation. The points indicate the means from four experiments. The lines are calculated regression lines. The slope of the curves are 0.217 (male) and 0.373 (female).

0.50) obtained by Huang and McIntosh (2). These authors demonstrated that the difference in the transport of PAH between the sexes was due to the stimulating effect of testosterone and not to the depressing effect of estrogens (2). Harvey and Malvin (1) reported that adult male rats have a higher creatinine clearance than do females. Injections of testosterone to females 4 hr prior to experimentation caused an increase in creatinine clearance to the male value while estrogen was without effect (1). No detectable sex difference in creatinine clearance in man (10) or in transport of PAH in rabbits (unpublished observation) has been observed. Thus, the differences seen in rats may be species dependent.

To determine the specificity of the effect exerted by testosterone, the uptake of the organic base TEA was determined in renal cortical slices from male and female rats. Since organic bases are transported by a system similar to, but distinct from, those for organic acids a sex difference in base transport would indicate a nonspecific effect of testosterone on renal transport systems. Preliminary results showed that a statistically significant difference in the TEA S/M ratio for cortical slices from male and female rats did exist. Decreasing concentration of TEA in the medium magnified the difference between male and female uptake (Fig. 2). The magnification of the difference between the ratios at low TEA concentration indicates a more effective transport system in tissue from males. The TEA ratio in slices from male rats fell at high medium concentration, suggesting saturation of the transport mechanism.

Since the S/M ratio is based upon tissue wet weight, differences could occur if slice composition varied with sex. It was necessary, therefore, to measure total water content, extracellular space, intracellular space and protein content of the cortical slices. However, no difference between male and female was observed when these components were measured (Fig. 3). It was concluded from these observations that the difference in accumulation of PAH could not be attributed to a gross difference in slice composition.

Inasmuch as the S/M ratio is determined in a steady state this value truly represents the algebraic sum of uptake, retention within the tissue, and runout back into the bathing medium. Maximal rate of uptake occurs in the very early incubation times when intracellulary accumulated PAH is not yet concentrated enough to decrease the rate of uptake (8). Thus, if a difference exists in the maximal rate of uptake this should be seen during very short periods of incubation. A large difference between the rate of uptake of PAH for male and female rats was indeed observed (Fig. 5). This was evidenced by differences in both apparent K_m and V_{max}. The differences in V_{max} suggest that more transport sites exist in the male tissue. The higher K_m for male tissue might indicate that male tissue has a greater load capacity. Although the data presented suggest that the uptake of PAH in cortical slices follows the pattern of Michaelis–Menten kinetics, a strict interpretation in light of enzyme kinetics is not altogether valid. Intracellular factors which are unaccounted for at the present time may be playing a role which is unexplained by the methods being used.

The rate constant for runout from female tissue was higher than from the males, indi-

cating faster runout of PAH from female renal cortical tissue (Fig. 4). This was supported by a difference in half-time for runout between the sexes. Both the greater rate constant and the shorter half time for runout indicate that egress of PAH from female cortical slices is faster than from male slices. Since runout from male kidney slices is slower, PAH remains intracellularly longer. More intracellular PAH as a result of slower runout from male slices will contribute to higher PAH *S/M* ratios.

Summary. Accumulation (*S/M*) of PAH and TEA was significantly greater in renal cortical slices from male than female rats. The data suggest, therefore, that the stimulatory effect of testosterone on active secretion of organic anions is not specific, but reflects a more generalized trophic effect on the kidney. The sex difference observed in accumulation of PAH was shown to involve differences in initial uptake rate and runout. It is concluded that the greater PAH *S/M* ratio exhibited by renal cortical slices from male rats results from a greater rate of uptake which is complemented by a slower rate of runout of the anion.

1. Harvey, A. M., and Malvin, R. L., Amer. J. Physiol. **209**, 849 (1965).

2. Huang, K. C., and McIntosh, B. J., Amer. J. Physiol. **183**, 387 (1955).

3. Ferguson, M., J. Physiol. (London) **169**, 24 (1963).

4. Cross, R. J., and Taggart, J. V., Amer. J. Physiol. **161**, 182 (1951).

5. Smith, H. W., Finkelstein, W., Aliminosa, N., Crawford, L., and Graber, M., J. Clin. Invest. **24**, 388 (1945).

6. Weber, W. A., and Cairns, J. A., Can. J. Physiol. Pharmacol. **46**, 165 (1968).

7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

8. Farah, A., Frazier, M., and Stoffel, M., J. Pharmacol. Exp. Ther. **139**, 120 (1963).

9. Clark, J. M., "Experimental Biochemistry." Freeman, San Francisco (1964).

10. Harvey, A. M., Malvin, R. L., and Vander, A. J., Nephron **3**, 201 (1966).

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