Effect of Sex Hormones on Renal Transport of \( p \)-Aminohippuric Acid

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A renotrophic effect of testosterone or its propionate has been reported by many investigators, referring especially to enlargement of the kidney. However, Welch, Rosenthal and Duncan (1) gave testosterone to three normal female dogs for 4 or more days and found an increase in maximal tubular excretion of Diodrast (\( T_m^D \)) without significant effect on glomerular filtration rate or effective renal blood flow of these animals. This increase of \( T_m^D \) was reversible after the administration of this steroid was stopped. Estrogen was administered to one dog but failed to influence \( T_m^D \). These results reveal an increased functional capacity of the renal tubules to excrete Diodrast as a result of testosterone but not of estrogen administration. White and his associates found that hypophysectomy resulted in a decrease of maximal tubular transport of \( p \)-aminohippuric acid (\( T_m^{PAH} \)). This reduction in tubular function is probably due to a deficiency of a substance present in growth hormone (2-6). The question that arises is whether the effect of testosterone to stimulate \( T_m \) is direct or through the anterior pituitary. Since the kidney slice technique of Cross and Taggart (7) has been used satisfactorily by many investigators to study renal tubular transport of PAH in vitro, we propose to use this technique to study this problem in intact and hypophysectomized rats.

Experimental

Albino rats (Wistar-Purdue) of both sexes, 4-6 months old were used in this investigation, their weights ranging from 150-250 gm. The sexes were kept in separate cages and fed with Purina laboratory food. The animals were divided and treated as following.

Intact Rats. Group 1: the animals served as untreated intact controls; group 2: animals of both sexes were treated with 10 mg of testosterone propionate in 0.2 ml peanut oil intramuscularly every other day; group 3: animals of both sexes were treated with 0.4 mg of estrone suspension solution intramuscularly every other day; group 4: consisted of castrated controls. Castration was performed under light ether anesthesia. One hundred thousand units of procaine penicillin suspension were given by intramuscular injection to each animal after the operation; group 5: castrated animals. Males were treated with 0.4 mg of estrone suspension intramuscularly every other day; females were treated with 10 mg of testosterone propionate intramuscularly every other day.

Hypophysectomized Rats. Hypophysectomy on 50 healthy adult rats was performed by the parapharyngeal approach. Forty-eight hours after the operation castration was done on some of these animals. All hypophysectomized animals received an intramuscular injection of procaine penicillin suspension weekly, and were fed a diet high in vitamin C and carbohydrate. They were grouped into: group 1: hypophysectomized males and females served as controls; group 2: female rats were treated with 10 mg of testosterone propionate intramuscularly every other day; group 3: male rats received an intramuscular injection of 0.4 mg of estrone every other day; group 4: castrated male and female controls; group 5: castrated females were treated with testosterone, and castrated males were treated with estrone.

The animals were stunned by a blow on the head. Their kidneys were removed immediately and put in ice-cold Ringer’s solution. Cortical slices were made and put in Warburg flasks which contained 3 ml of acetate-Ringer solution with 0.075 mM \( p \)-aminohippuric acid (PAH). The procedure of slicing the kidney, the preparation of the acetate-Ringer solution and the analytical technique used have been reported previously by the authors (8). After 5 minutes oxygenation the flasks were incubated in a water bath at constant temperature of 25°C. One hour later, the slices were removed from the flask, blotted, weighed, and homogenized with 5% trichloroacetic acid solution. The rate of PAH uptake by the slices is calculated as \( \mu l. O_2/hr/mg \) of wet tissue (final weight) (\( Q_o \)).

Results

Non-Hypophysectomized Rats. Table 1A contains a summary of data covering the number and type of rat used, drug given, PAH \( S/M \) ratios, \( Q_o \)'s and kidney size on the non-hypophysectomized group of animals.

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Table 1. Effect of sex hormones on PAH uptake, QO2 and kidney size

<table>
<thead>
<tr>
<th>Group, Sex, No.</th>
<th>Treatment</th>
<th>PAH S/M Ratio</th>
<th>QO2 μL O2/mg/hr.</th>
<th>Kidney Size, % of Body Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug</td>
<td>Doses given</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intact</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, M, 8</td>
<td>None</td>
<td>15.4±1.0*</td>
<td>2.18±0.099*</td>
<td>0.706</td>
</tr>
<tr>
<td>F, 13</td>
<td>None</td>
<td>8.4±0.50</td>
<td>2.18±0.685*</td>
<td>0.707</td>
</tr>
<tr>
<td>2, M, 5</td>
<td>Testosterone 4</td>
<td>14.9±1.65</td>
<td>2.14±0.044</td>
<td></td>
</tr>
<tr>
<td>F, 6</td>
<td>Testosterone 4</td>
<td>14.6±0.84</td>
<td>2.20±0.053</td>
<td></td>
</tr>
<tr>
<td>3, M, 6</td>
<td>Estrone 4</td>
<td>10.1±0.79</td>
<td>2.31±0.033</td>
<td></td>
</tr>
<tr>
<td>F, 6</td>
<td>Estrone 4</td>
<td>7.3±0.66</td>
<td>2.00±0.049</td>
<td></td>
</tr>
<tr>
<td><strong>Castrated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4, M, 10</td>
<td>None</td>
<td>8.6±0.43</td>
<td>2.04±0.09</td>
<td>0.702</td>
</tr>
<tr>
<td>F, 8</td>
<td>None</td>
<td>6.6±0.33</td>
<td>1.85±0.13</td>
<td>0.698</td>
</tr>
<tr>
<td>5, M, 13</td>
<td>Estrone 9-11</td>
<td>7.9±0.59</td>
<td>1.92±0.052</td>
<td>0.797</td>
</tr>
<tr>
<td>(x)</td>
<td>Testosterone 9-11</td>
<td>8.8±0.97</td>
<td>1.69±0.0097</td>
<td></td>
</tr>
</tbody>
</table>

**Non-castrated**

1, M, 5 | None | 6.3±1.1* | 1.45±0.04* | 0.552
F, 5 | None | 6.2±0.20 | 1.36±0.06 | 0.551
2, F, 4 | Testosterone 12 | 3.5±0.05 | 1.52±0.00 | 0.500
3, M, 4 | Estrone 12 | 3.0±0.04 | 1.20±0.10 | 0.59

**Castrated**

4, M, 4 | None | 5.8±0.38 | 1.46±0.05 | 0.572
F, 4 | None | 5.2±0.57 | 1.46±0.15 | 0.544
5, M, 8 | Estrone 12 | 3.5±0.57 | 1.34±0.26 | 0.544
F, 6 | Testosterone 12 | 3.0±0.86 | 1.07±0.05 | 0.703

* Standard error.

**Group 1, intact controls.** Data showed that the male kidney slices had greater activity for PAH uptake than did the female kidney. For example, the average S/M ratio for male kidney slices was 15.4, while that of the female was 8.4. This difference is not related to the size of the kidneys and the oxygen consumption of the slices.

**Group 2, testosterone treatment.** Four doses of testosterone propionate given to six female rats produced an S/M ratio of 14.6, compared to the value of 8.4 observed in the female control group. QO2 was not significantly different than that observed in the female controls. On the other hand, the same doses of testosterone given to 5 male rats produced a metabolic rate (QO2) slightly greater, but statistically not significantly greater, than that of control males, and an S/M ratio essentially the same as that of control males.

Administration of one, instead of four doses of testosterone propionate to five female rats (not included in the table) resulted in S/M ratios and in QO2 values statistically no different than those of control females.

**Group 3, estrone treatment.** In the males, estrone treatment produced an S/M ratio of 10.1, significantly lower than that of control males, and a QO2 value of 2.31, significantly higher than that of the controls.

In the six females, estrone treatment resulted in an S/M ratio and QO2 value slightly lower, but statistically not significantly lower, than that of the control females.

**Group 4, castrated animals.** In a group of 10 castrated male rats the kidney slices failed to concentrate PAH to the same extent as the non-castrated controls (S/M = 8.6; QO2 = 2.04). In the group of eight castrated females, the kidney slices concentrated PAH to a slightly lesser extent than did the kidneys of intact controls (S/M = 6.6). The QO2 of the slices was significantly reduced to 1.85 when compared to the controls.

**Group 5, castrated animals treated with testosterone or estrone.** Nine to eleven doses of estrone were administered to 15 castrated males. Kidney slices of 13 of these animals had a low S/M ratio and metabolic rate, the average being 7.7 and 1.02, respectively. The other two animals had higher S/M ratios, averaging 14.8. Nine to eleven doses of testosterone propionate were given to 21 castrated females. Kidney slices of 19 of these animals had an average S/M ratio of 15.9 and QO2 of 2.38; in the other two animals S/M ratio did not increase.

**Hypophysectomized Rats.** Studies on a group of hypophysectomized controls showed that the kidneys of these animals were markedly atrophied. The ability of the kidneys to transport PAH and the metabolic rate of the slices were definitely lower than those of the intact controls. As shown in table 1B, there was little difference between the S/M ratio and QO2 of the male and the female. An average S/M ratio of the hypophysectomized males was 6.3 and of the hypophysectomized females was 6.1. An average QO2 of males was 1.45, of females 1.36. Castration did not cause a further decrease in the size of the atrophied kidneys, in the S/M ratio or in the oxygen consumption by the slices. An average S/M ratio for castrated hypophysectomized males and females was 5.8 and 5.2, respectively. The QO2 was 1.48 for males, and 1.46 for
females. Statistical analysis showed that these data were not significantly different from those of the non-castrated hypophysectomized animals.

In a group of non-ovariectomized and ovariectomized hypophysectomized rats, testosterone propionate administration did increase the size of the atrophied kidneys from 0.535% of body weight to 0.7% of body weight in non-ovariectomized and from 0.547% to 0.703% in ovariectomized animals, but did not increase the rate of accumulation of PAH.

Estrone administration produced a slight increase in the size of the atrophied kidneys of both non-castrated and castrated hypophysectomized males. The size of the kidneys increased from 0.522% of the body weight to 0.59% in non-castrated, and from 0.572% of body weight to 0.644% in castrated. Estrone caused a further depression of the PAH transport and metabolism of the kidney slices.

DISCUSSION

The literature reveals that the sex hormones have a definite effect on the growth and size of the kidneys. Ovariectomy increases the growth rate of kidneys in rats (9) and administration of estrogenic substances to rats will cause a decrease of kidney weight (10) or cyst-like degeneration in renal cortex and medulla (11). A decrease of the kidney size is observed in castrated male rats (12–14). Testosterone and its propionate enlarge the kidneys of intact rats (10, 14) and castrated rats (12–16). This obviously indicates that the male sex hormone exerts a stimulating, and the female hormone a depressing effect on the kidneys.

As far as the renal tubular function to transport PAH is concerned, our results show that the kidney of the normal male rat has a much higher tubular excretory capacity than the kidney of the normal female, although the size and the metabolic rate of the kidney is the same in the two sexes. Castration causes a fall of renal tubular function and rate of metabolism by the kidney slices. Testosterone and its propionate enlarge the kidneys of intact rats (10, 14) and castrated rats (12–16). This obviously indicates that the male sex hormone exerts a stimulating, and the female hormone a depressing effect on the kidneys.

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From the results shown in table 1A it is obvious that the $Q_{O_2}$ of the slices is increased after administration of testosterone or estrone and is decreased after castration. This can be explained in that both the male and female sex hormones have anabolic effect on the tissue (17). This anabolic effect on kidneys is not related with the tubular capacity to transport PAH.
Selye studied the effect of testosterone on intact and hypophysectomized rats (18) and found that this hormone increased the kidney sizes of both groups of animals but failed to restore atrophied kidneys of hypophysectomized animals to normal even when follicle-stimulating hormone, interstitial-cell stimulating hormone or adrenocorticotrophic hormone was given in combination with testosterone. Therefore he concluded that there must be an 'additional' hormone in the pituitary, besides the gonadotrophic and corticotrophic hormone, for the maintenance of normal kidney size. The result of our study on hypophysectomized rats has shown that the ability to transport PAH and the metabolic rate of the kidney slices were markedly reduced after hypophysectomy. No further reduction ensues following the removal of the gonads and neither the rate of PAH uptake nor $Q_{O_2}$ are restored by the administration of testosterone or estrone, but this reduction of PAH uptake can be restored by the administration of growth hormone as demonstrated by Farah and his co-workers (19). These data suggest strongly that with respect to renal transport of PAH the effect of the hypophysis is much more prominent than the stimulating effect of testosterone. Although the anterior pituitary gland, by the elaboration of gonadotrophic hormone, regulates to a large extent the development of the gonads, the possibility that the hypophyseal effect on renal tubular function is mediated through the secretion of sex hormones is very remote. From their in vivo studies on dogs, White and his co-workers concluded that the effect of the hypophysis on the kidney is probably direct and not through the thyroid or adrenocortical hormones (4, 5).

As shown by our results, testosterone administration did not restore PAH uptake by the kidney slices of hypophysectomized rats. It did increase the size of the kidney to normal even in the absence of hypophyseal hormones. Selye's conclusion that the 'renotropic' effect of testosterone is probably direct and is not mediated through the pituitary is true only with respect to kidney size, but not with respect to tubular function.

**SUMMARY**

The uptake of PAH by kidney slices of male rats is greater than that of female rats. Estrone administration or castration causes a decrease in this tubular function in the male. Testosterone increases PAH uptake by kidney slices from intact and ovariectomized female rats. A marked reduction of PAH uptake and oxygen consumption is observed in kidney slices from hypophysectomized rats. These functions are not reduced further by castration. In hypophysectomized rats, testosterone increases the size of the kidneys and oxygen consumption, but does not increase PAH uptake of kidney slices.

**REFERENCES**