EFFECT OF HYPOPHYSECTOMY ON p-AMINOHIPPURATE 
TRANSPORT KINETICS IN RAT RENAL CORTICAL SLICES

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(Received 12 October 1976)

SUMMARY

Recent work on kidneys from hypophysectomized (hypox) rats has shown atrophy of the 
proximal tubules with no effects on the other parts of the nephron. We carried out experi-
ments to determine whether the reduction of $p$-aminohippurate (PAH) is consistent with 
structural changes in the proximal tubule of hypophysectomized rats. Initial velocities 
of PAH uptake by renal cortical slices were found to be constant over 30 min of incubation 
at concentrations of PAH up to 0.5 mmol/l for both control and hypox animals. Using 
kinetic analysis, it was found that both maximal velocity, $V_{\text{max}}$, and the Michaelis constant, $K_m$, were reduced in hypox animals, the relative reduction being similar for both parameters. 
Comparison between high Na (100 mmol/l) and low Na (6 mmol/l) media indicated that in 
both control and hypox rats, $V_{\text{max}}$ was significantly lower in low Na medium than in high 
Na medium, whereas $K_m$ was not changed. Efflux of PAH from pre-loaded tissue also showed 
a reduction in hypox animals. These results may indicate that hypophysectomy alters the 
capacity of PAH transport in renal cortical slices by (1) reducing the effective transport 
area or sites, and (2) by changing carrier-substrate affinity.

INTRODUCTION

Previous work by Farah, Koda & Frazer (1956) has shown that there is a decrease in the 
transport of $p$-aminohippurate (PAH) in renal slices after hypophysectomy. These authors 
attributed this change to the impaired secretion of certain hormones such as thyroxine and 
growth hormone, since hormonal therapy restored PAH transport to near normal levels. 
The mechanism by which these hormones alter PAH transport, however, was not elucidated 
in this study.

Recent work on kidneys from hypophysectomized (hypox) rats (Evan, Simone, Solomon 
& Loker, 1972) has shown atrophy of the proximal tubule with no apparent damage to 
the rest of the nephron. Most notable of these changes in cells of the proximal tubules are 
a decrease in cell volume, membrane area, number of microvilli and prominence and occurrence 
of basilar infoldings.

Since the uptake of PAH by mammalian renal slices is probably mediated by a carrier 
system located in the peritubular membrane (Huang & Lin, 1965; Park, Yoo & Hong, 
1971), a decrease in basilar infoldings with a consequent loss of peritubular membrane area 
may constitute a decrease in available carriers to mediate the transfer of PAH.

We therefore undertook kinetic studies of PAH transport to determine whether reduction 
of PAH transport is consistent with structural changes in the proximal tubule of hypox 
rats. If hypophysectomy resulted in a loss of membrane carriers without change in the 
nature of the transport system, then a reduction in maximum transport rate ($V_{\text{max}}$) should 
occur without altering the Michaelis constant ($K_m$).
METHODS

Normal and hypox male Sprague–Dawley rats were obtained from the Charles River Breeding Laboratory (Wilmington, Massachusetts, U.S.A.) and maintained for 8 weeks in the laboratory before starting the experiments. The rats were killed by a blow on the neck, and the kidneys were immediately removed, decapsulated, and placed in an ice-cold, oxygenated slicing medium (composition in mmol/l: Na acetate, 5; KCl, 10; CaCl2, 1·5; choline chloride, 95; Tris–HCl, 40), pH 7·6, at 25 °C similar to that described by Gerencser, Park & Hong (1973). Renal cortical slices approximately 0·4–0·5 mm thick and weighing approximately 100 mg were cut using a Stadie–Riggs tissue slicer and placed in the above medium. In the influx studies, slices were transferred to an Erlenmeyer flask containing 10 ml of an appropriate incubation medium equilibrated at 25 °C in a Dubnoff shaker.

After addition of 0·1 μCi [14C]PAH (NEN) and 1·0 μCi [3H]inulin (Amersham/Searle), incubations were carried out for selected periods of time under constant bubbling of oxygen. To estimate passive uptake, one series of flasks contained 1 mm-iodoacetic acid and was gassed with nitrogen. Active uptake into the cell was computed by subtracting the uptake value of the metabolically inhibited slices from that of the uninhibited slices. In most studies, ionic composition of the incubation medium was the same as that of the slicing medium except that choline chloride was replaced by an equivalent amount of NaCl. When the effect of low Na concentration was studied, the incubation medium had the same composition as the slicing medium. Upon completion of incubation, slices were removed from the medium, rinsed in PAH-free medium, blotted on filter paper and weighed. The tissue was then placed in distilled water overnight to leach out inulin and PAH from the tissue. p-Aminohippurate concentration was determined on both the leaching and final incubation media by the method of Smith, Finkelstein, Aliminosa, Crawford & Graber (1945). Radioactivities were determined using a liquid scintillation spectrometer (Packard Tri-Carb) with samples dissolved in Phase Combining System (Amersham/Searle). Cellular water space was assessed by subtracting [3H]inulin space from total tissue water content. Total tissue water was determined separately by taking the difference between wet and dry tissue weights.

The efflux of PAH from pre-loaded slices was studied also. Slices were first incubated for 60 min in a high Na medium (100 mmol/l) with 0·1 m-PAH and 0·2 μCi [14C]PAH/ml. Slices were removed, quickly rinsed, blotted and transferred to beakers containing 10 ml PAH-free medium. Tissue was incubated either aerobically or anaerobically. At 3 min intervals, aliquots of the medium were removed for sampling of radioactivity. At the end of a 15 min period the slices were removed, rinsed, blotted, and placed in distilled water to leach out [14C]PAH. Radioactivity in the leaching solution was determined as described above. The total counts effluxed into the medium and the counts remaining in the tissue were combined to determine initial radioactivity in the slices. The percentage of radioactivity remaining in the tissue at the end of each efflux period was plotted as a function of time using a semi-logarithmic plot. The slope of this line represents the rate of efflux.

Curves were fitted using least squares procedures, and comparisons of means were evaluated using Student’s t-test.

RESULTS

To determine over what range of time PAH uptake was linear, PAH accumulation as a function of time was measured. Experiments were carried out in media with or without the addition of iodoacetate and nitrogen at a constant PAH concentration of 0·1 or 0·5 mmol/l. Subtracting the accumulation of PAH in the poisoned slice from that obtained from the slice in the normal medium during that period gives the net active accumulation for that
Fig. 1. Time course of active p-aminohippurate accumulation (mmol/kg wet tissue) by rat kidney slices of normal (●) and hypophysectomized (▲) rats. Each point represents the mean of five slices. Vertical bars indicate ±S.E.M.

period. Slices incubated in high Na medium (100 mmol/l) at a PAH concentration of 0.5 mmol/l gave the results as expressed in Fig. 1. Active PAH uptake appeared to be linear up to 30 min for slices from both control and hypox animals. Since any period between 0 and 30 min would reflect the initial transport rate, the 30 min time was selected for incubation during the kinetic investigation.

The next group of experiments was designed to determine the effect of hypophysectomy on the uptake of PAH. Figure 2 shows active PAH uptake into the cell as a function of PAH concentration in 100 mm-Na medium. p-Aminohippurate uptake increased curvilinearly in hypox as well as control rat tissue and tended to be saturated at high concentrations of PAH. Data were subjected to Hofstee analysis to determine various kinetic parameters. As illustrated in Fig. 3 and summarized in Table 1, both $V_{max}$ and $K_m$ were significantly lower in the hypox rat than in the control animal.

Since numerous past studies (Vogel & Stoeckert, 1966; Chung, Park & Hong, 1970; Gerencser et al. 1973) have shown a dependence of active transport of organic solutes on the concentration of Na, the next group of experiments was designed to determine whether Na concentration in the medium affects the uptake of PAH by rat kidney obtained from hypox rats. Figure 4 shows uptake of PAH in a low Na medium (6 mmol/l). p-Aminohippurate uptake again increased curvilinearly in both control and hypox rats with an increase in PAH concentration in the medium. The $V_{max}$ value for control rats was greater than that in the hypox rats (Fig. 5 and Table 1). However, comparison between high Na (100 mmol/l) and low Na (6 mmol/l) media indicated that both in control and hypox rats, $V_{max}$ was significantly lower in low Na medium than in high Na medium, whereas $K_m$ was not changed.
Fig. 2. Rate of active uptake of $p$-aminohippurate (PAH) (mmol/kg wet wt/30 min) by rat kidney slices in the final medium concentration (100 mM-Na medium). Each point represents the mean of five slices of normal tissue (○) and four slices of hypophysectomized tissue (▲). Vertical bars indicate ± S.E.M.

Fig. 3. Hofstee kinetic analysis of $p$-aminohippurate (PAH) uptake by slices of kidney tissue from normal (○) and hypophysectomized (▲) rats incubated in 100 mM-Na medium. The active uptake concentration of PAH for 30 min ($V_\text{u}$) is plotted as a function of the ratio of cell to medium [PAH], $V_u/[S]$. The intercept represents $V_\text{max}$ of uptake. The slope of the line represents negative $K_m$. $V_\text{max}$ and $K_m$ are in mmol/kg wet tissue/30 min and mmol/l respectively.
Table 1. Kinetic parameters for uptake of p-aminohippurate by normal and hypophysectomized (hypox) rat kidney slices (means ± S.E.M.)

<table>
<thead>
<tr>
<th>100 mM-Na</th>
<th>No. of slices</th>
<th>V_max (mmol/kg/30 min)</th>
<th>P value</th>
<th>K_m (mmol/l)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>2.55±0.14</td>
<td>&lt; 0.005</td>
<td>0.25±0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Hypox</td>
<td>4</td>
<td>1.00±0.16</td>
<td></td>
<td>0.14±0.04</td>
<td></td>
</tr>
<tr>
<td>6 mM-Na</td>
<td>5</td>
<td>0.52±0.13</td>
<td>&lt; 0.0125</td>
<td>0.25±0.07</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.18±0.05</td>
<td></td>
<td>0.10±0.06</td>
<td></td>
</tr>
<tr>
<td>Hypox</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

V_max, Maximum velocity. K_m, Michaelis constant.

**DISCUSSION**

In kinetic analysis of transfer, the term V_max is determined by the number of transport sites, the probability that a substrate molecule will dissociate from a carrier site in a given time, and the rate of turnover of a carrier across the membrane. The concentration of substrate for one-half saturation of the transport mechanism, K_m, is determined by the affinity, or more precisely by the rates of association and dissociation of the carrier and substrate (Neame & Richards, 1972).

The results of the efflux experiments using slices pre-loaded with PAH are shown in Fig. 6. Statistical analysis of the efflux curves is indicated in Table 2. Slices incubated aerobically showed a decrease in the efflux of PAH from control tissue as compared with hypox tissue. With the addition of iodoacetate and nitrogen to minimize or eliminate re-uptake of PAH, there was a decrease in the efflux of PAH in hypox rat tissue as compared with the control tissue over the period studied.
Fig. 5. Hofstee kinetic analysis of \( p \)-aminohippurate (PAH) uptake by slices of kidney tissue from normal (\( \bullet \)) and hypophysectomized (\( \triangle \)) rats incubated in 6 mM-Na medium. The active uptake concentration of PAH for 30 min (\( V_a \)) is plotted as a function of the ratio of cell to medium [PAH], \( V_a/[S] \). The intercept represents \( V_{\text{max}} \) of uptake. The slope of the line represents negative \( K_m \). \( V_{\text{max}} \) and \( K_m \) are in mmol/kg wet tissue/30 min and mmol/l respectively.

Fig. 6. Efflux of \( p \)-aminohippurate from pre-loaded kidney slices from normal (\( \bullet \)) and hypophysectomized (\( \triangle \)) rats incubated in 100 mM-Na medium either in the presence of \( O_2 \) (solid lines) or iodoacetate and \( N_2 \).
Hypophysectomy and renal PAH transport

Table 2. Efflux of p-aminohippurate from pre-loaded kidney slices of normal and hypophysectomized (hypox) rats (means ± S.E.M.)

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>No. of slices</th>
<th>Efflux rate (mmol/kg/min)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0.27±0.001</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Hypox</td>
<td>5</td>
<td>0.34±0.001</td>
<td></td>
</tr>
<tr>
<td>Iodoacetic acid+N₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0.09±0.005</td>
<td>&gt; 0.0025</td>
</tr>
<tr>
<td>Hypox</td>
<td>5</td>
<td>0.06±0.002</td>
<td></td>
</tr>
</tbody>
</table>

In the present study, both \( V_{\text{max}} \) and \( K_m \) for PAH transport in renal slices were reduced in hypox rats (Figs 3 and 5 and Table 1). The mechanism for these changes is not entirely understood at present. The reduction of \( V_{\text{max}} \) may be primarily due to a decreased peritubular membrane area of the proximal tubule cell. Hence, transport sites per unit mass of kidney tissue in hypox rats are reduced, as observed by Evan et al. (1972). However, alteration of the transport system itself would also be involved. In kidney slices (Park et al. 1971) and in other tissues (Neame & Richards, 1972), a substrate which has a lower \( K_m \) (thus higher carrier affinity) than another substrate shows a relatively higher rate of transport at low concentrations and a relatively lower rate of transport at high concentrations, thus lower \( V_{\text{max}} \). According to Forster (1967), this is because when a carrier is nearly loaded it is the dissociation of the carrier–substrate complex rather than its formation that determines overall transport of free substrate. Reduction of \( K_m \) in hypox rats would therefore indicate that the transport system is altered, such that the carrier–substrate affinity is increased and at the same time \( V_{\text{max}} \) is reduced. This fact also emphasizes that kidney function changes in an adaptive way after hypophysectomy. With relatively fewer transport sites, an increase in the substrate affinity could enhance the efficiency of the physiological secretion of organic acids.

Previous work with hormone therapy in hypox rats has resulted in restoration of the PAH transport capacity in renal slices (Farah et al. 1956). This observation can be interpreted as indicating that thyroid and growth hormones are mainly involved in restoration of the effective membrane area rather than an increase in the carrier–substrate affinity. Since thyroxine therapy in normal rats reduces depression of PAH uptake by renal slices (Nepomuceno & Little, 1964), thyroxine treatment probably reduces carrier–substrate affinity.

Reduction of \( V_{\text{max}} \) with no change in \( K_m \) in low Na medium (Table 1) is consistent with previous findings in rabbit renal slices (Gerencser et al. 1973). Similar Na dependence in hypox rats (Table 1) suggests that the basic mechanisms of PAH transport may be unaltered by hypophysectomy.

Efflux studies are also consistent with the view that the major effect is on membrane area or number of carriers. It has been suggested that efflux takes place by way of transporting sites (Kinter & Cline, 1961), but this view is not held by all (Kinter, 1966; Ross & Farah, 1966). In any event, the reduced efflux rates found in metabolically inhibited hypox rat kidney slices can be interpreted in terms of a reduced number of pathways available for efflux. The failure to find this pattern in non-metabolically inhibited slices probably reflects re-uptake by the still functional tissue. A higher rate of uptake in control animals would serve to reduce the loss of PAH.

This work was supported by a grant from the National Science Foundation, BMS 72-02344, and a training grant from the National Institutes of Health, HL 05633.
REFERENCES


