EFFECTS OF CORTISOL ON THE HENLE'S LOOP OF THE ADRENALECTOMIZED RAT'S KIDNEY

FUMINORI SAKAI AND YOSHIMICHI MURAYAMA
Department of Pharmacology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo

Received for publication June 19, 1970

The experiment to be reported is an attempt to investigate the mechanism of action of cortisol in the Henle's loop.

Male albino rats weighing 170 to 270 g were adrenalectomized and given free access to 0.9% saline. Six to seven days after adrenalectomy, five milligram of cortisol (alcohol free) was injected subcutaneously for 2 days previously. Additional cortisol (5 mg) was injected subcutaneously 60 minutes prior to the start of the experiment.

The method of this experiment was almost same as that previously reported (1). A proximal tubule of anesthetized rat (Nembutal 30 mg/kg i.v.) was punctured with a perfusion pipette and a sample was collected from the first part of the distal segment of the surface of the kidney. Ringer's solution (NaCl; 147 mM KCl 4.02 mM, CaCl₂; 2.7 mM) containing inulin (0.15–0.20%) and lissamine green 0.05%) was used as perfusate and perfusion rate was 19 nl/min. The osmolality of this solution was 302 mOsm/L. The osmolality, Na and inulin concentration of the samples were measured with a micro-osmometer, an ultramicroflamephotometer (Erma Model 677) and micro-fluorophotometer (AMINCO) (2). These values were shown as a TF/p.

The data of ureteral urine osmolality are the mean values of the samples which were collected 100 to 200 minutes after anesthesia.

The ratios of the Na concentration and osmolality in adrenalectomized rats were almost same as those previously reported and tended to decrease by the application of cortisol (from 0.69 to 0.50 (Na), from 0.80 to 0.66 (osm)). However, the ratios of the inulin concentration were not affected with cortisol. The net sodium reabsorption was calculated by a following equation. 

\[ N_{\text{areab.}} = V_p \cdot \left( \frac{\text{TF}}{p} \right)_N - \left( \frac{\text{TF}}{p} \right)_\text{in} \]

As shown in Table 1, the values of \( N_{\text{areab.}} \) increased with cortisol, (from 1.48 to 1.88) and the differences were statistic significant.

It has been reported that cortisol may affect directly on the water permeability of the tubular membrane (3). At the end of our each experimental series latex fluid was reinjected into the perfused tubular lumen and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Rats</th>
<th>(TF/P)_osm</th>
<th>(TF/P)_Na</th>
<th>(TF/P)_in</th>
<th>( N_{\text{areab.}} )</th>
<th>( U_{\text{osm}} ) (mOsm/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomy</td>
<td>9</td>
<td>0.80 (24)</td>
<td>0.69 (20)</td>
<td>1.49 (18)</td>
<td>1.48 (16)</td>
<td>1048</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.012</td>
<td>± 0.016</td>
<td>± 0.077</td>
<td>± 0.25</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomy +</td>
<td>5</td>
<td>0.66* (19)</td>
<td>0.50* (18)</td>
<td>1.51 (18)</td>
<td>1.88* (17)</td>
<td>1672*</td>
</tr>
<tr>
<td>Cortisol 5 mg x 3</td>
<td></td>
<td>± 0.019</td>
<td>± 0.017</td>
<td>± 0.066</td>
<td>± 0.06</td>
<td>± 94</td>
</tr>
<tr>
<td>days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* means statistic significant, P<0.01
Numbers in parenthesis present the numbers of the sample.
Standard errors of each values were calculated.

The data of urteral urine osmolality are the mean values of the samples which were collected 100 to 200 minutes after anesthesia.

Supported by NIH grant AM09742.
the punctured site of the proximal and distal segments were measured with a method of microdissection.
The punctured points of the proximal and distal tubules were 40 to 55% and 15 to 55% of these segments.

The samples collected from distal tubule contained lissamine green. However, the effect of this dye on the
determination of the inulin concentration was negligible in a concentration which was used in this experiment (4). It is clear that the GFR of the kidney of the adrenalectomized rat decreased. But, our data is
independent on these changing of GFR, because the glomerular fluid was blocked and the tubular segments
were perfused with a constant flowing volume. Cortisol may affect on the water permeability more distal
parts than 55% of the distal tubules.

REFERENCES
1) Murayama, Y., Suzuki, A., Tadokoro, M. and Sakai, F.: Jap. J. Pharmac. 18, 518 (1968); 2) Vurek,

MICRO-DETERMINATION OF INULIN CONCENTRATION IN
A SOLUTION CONTAINING LISSAMINE GREEN

ICHIRO IKEZAWA, AKIYOSHI SUZUKI
AND FUMINORI SAKAI

Department of Pharmacology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo

Received for publication June 19, 1970

Microdissection technique has contributed to our understanding of renal physiology. Particularly the
microphotometric determination of the inulin concentration in tubular fluid collected through a micropuncture
technique is a key point for the analysis of the water movement across the tubular cell membrane.
Hilger et al. (1). Vurek and Pegram (2) have developed their methods. In the experiment to be reported
now was attempted to investigate the effect of lissamine green on the determination of inulin concentration,
because the tubular fluid contained a dye of lissamine green which was injected into the tubular lumen, in
order to identify a tubular segment. A method of microfluorophotometer was applied. In the first experi-
mental series the test solution were prepared as follows: Four test solution, containing 1, 2, 3, 4 and 5 mg/ml
inulin in distilled water were prepared and lissamine green was added further to each solution (See Fig. 1).

Ten nano liter of the test solution was mixed with 2.5 µl of Dimedone solution (5 mg/ml in phosphoric
acid) and measured the inulin concentration using AMINO type fluorophotometer following the Vurek's
method. The results were summarized in Fig. 1-a. The values of each series of the test solution showed a
linear correlation with a serial concentration of inulin and the differences of the values between each other
were not observed.

In the second series of the experiments, the same test solutions were used, but 25 nl of each test solution
was mixed with 2.5 µl Dimedone solution. The results were summarized in Fig. 1-b. As shown in this
Fig., the values decreased according to the increasing of the concentration of dye. The high concentration

Supported by NIH grant AM09742.