Inhibitory Effect of 6-Aminonicotinamide on the Renal Transport of Para-Aminohippurate in the Rat

Jun-ichi SUDO, Atsushi ISHIHARA and Tsuneyoshi TANABE
Department of Toxicology, Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido 061-02, Japan

Accepted September 6, 1984

Abstract—In this study, we investigated the effect of 6-aminonicotinamide (6-AN), a typical potent inhibitor of the pentose phosphate pathway, on the renal transport of para-aminohippurate (PAH) in the rat. The contents of adenosine-triphosphate (ATP) and 6-phosphogluconate (6-PG) in the kidney were measured at intervals of 2 hours after the administration of 6-AN (75 mg/kg body weight, i.p.). It was found that the 6-PG content in the kidney rapidly increased and reached a plateau at the fourth hour after the administration, with this level being maintained up to the eighth hour. In contrast, the ATP content was found to remain normal up to the sixth hour, after which it significantly decreased as time elapsed. Furthermore, additional experiments were carried out by loading the rat with a high concentration of PAH solution at 6 hours after the administration of 6-AN. The renal tubular secretion maximum for PAH was significantly depressed in the 6-AN group in comparison to the control. These results suggest that this depression in renal PAH secretion capacity was partially due to the inhibition of the pentose phosphate pathway in the kidney, but not due to the change of renal ATP level.

6-Aminonicotinamide (6-AN) is known to be a 6-amino derivative of nicotinamide. Coper and Neubert (1) reported that this agent was a potent inhibitor of the oxidoreductases which were dependent on nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Furthermore, it is known that 6-AN inhibits the pentose phosphate pathway in the kidney, and it also exhibits an inhibitory effect on sodium transport in the kidney (2).

The present authors hypothetically considered that the renal active transport systems could be affected when the renal glycolytic system is depressed because the energy for active transport could be speculated to be partially supported by glycolysis (3, 4). Accordingly, using 6-AN which is known as a typical inhibitor in the pentose phosphate pathway in glucose metabolic systems, the authors investigated the relationship between the renal transport of para-aminohippurate (PAH) and the depression of the pentose phosphate pathway caused by administration of 6-AN in rat kidney, with the intent of elucidating the above-described hypothesis.

Materials and Methods

Studies were performed in male Wistar rats weighing 200–250 g. The rats were allowed free access to water and fed a standard rat pellet diet prior to the study. They were divided into two groups, one being administered 6-AN in saline (75 mg/kg body weight, i.p.) and the other (the control group) being administered an equivalent volume of saline.

In experiments to investigate changes of adenosine-triphosphate (ATP) and 6-phosphogluconate (6-PG) in the kidney, the rat was given free access to water and diet after the administration of 6-AN, and each rat was kept in a separate cage. For these measurements, the kidney was removed from the rat at intervals of 2 hours after the
administration: The rat was anesthetized with ether, and the left kidney was exposed through a ventral incision. The left kidney was removed and immediately put into liquid nitrogen. It was homogenized, for deproteinization, using a Polytron (Kinematica Co., Switzerland) with 2 ml of cold 6% perchloric acid per 1 g of the tissue. After keeping it for 10 min at 4°C, the homogenate was centrifuged for 15 min at 3000 r.p.m., and the supernatant was taken. An equivalent volume of cold 10% potassium bicarbonate solution was added to the supernatant to adjust its pH to about 7.4. After recentrifugation of this supernatant for 15 min at 3000 r.p.m., the obtained supernatant was used to measure the ATP and 6-PG contents. The content of ATP was measured using ATP Test-Combination according to the method of Bucher (5), and the content of 6-PG was measured by the method of Haid (6) with the modification described by Horecker and Smyrniotis (7). For absorbance analyses, a Hitachi-320 (Hitachi Co., Tokyo, Japan) spectrophotometer was used.

In experiments to investigate the renal tubular secretion maximum for PAH in the kidney, the rats were anesthetized intraperitoneally with 50 mg/kg body weight of sodium pentobarbital and then intubated for free respiration, after which a left femoral vein was catheterized with a polyethylene tube (PE-50). These operations were started at the fourth hour after the administration of 6-AN. Throughout each experiment, saline containing 12% inulin and 0.05% PAH was injected at a rate of 2 ml/kg body weight as a prime, followed by infusion of saline containing 0.08% inulin and 0.05% PAH at a rate of 0.5 ml/kg body weight/min. An equilibration period of at least 60 min was allowed. The left ureter was approached retroperitoneally and was catheterized with a polyethylene tube (PE-10) (8). Urine was collected every 5 min, with tail blood being taken at intervals of 10 min. For the loading of PAH, 0.5% PAH in the above described infusion solution was infused at the same rate. This load of PAH saline solution was begun at 6 hours after the administration of 6-AN, as described in the Results and Discussion. At the conclusion of the experiment, the wet weight of the left kidney was measured after a ventral incision. Sodium and potassium in urine and plasma were measured using a flame photometer FLAME 30 C (Jasco Medical Instruments Inc., Tokyo, Japan). Inulin in urine and plasma was analyzed by the anthrone method of Führ et al. (9). PAH concentrations in urine and plasma were analyzed according to the method of Smith et al. (10).

For calculation of the renal tubular secretion maximum for PAH (TmPAH), the ratio of free PAH concentration to the total one in plasma was measured under the same condition as described above. Heparinized blood was taken from the abdominal aorta through a ventral incision 40 min after the start of PAH loading. At this time, the plasma PAH concentration reached a level of more than 20 mg/dl. It was then centrifuged for separation of plasma. The obtained plasma was ultrafiltered using Immersible CX-30 Ultrafilters (30000 molecular weight cut-off, Millipore Co., Bedford, Massachusetts, U.S.A.), and the plasma free PAH concentration and the total PAH concentration were measured. The ratio of free PAH concentration to the total one, as represented by means±standard error (S.E.), was 0.901±0.017 (number of experiments=6). The TmPAH was thus calculated, using this coefficient of 0.901, from the following formula:

\[ T_{mPAH} = \frac{U_{PAH} \times V - 0.901 \times P_{PAH} \times C_{in}}{U_{PAH} \times (mg/ml)} = \text{urinary PAH concentration} \]
\[ V \times (ml/min/g \text{ wet weight of kidney}) \]
\[ P_{PAH} \times (mg/ml) = \text{plasma PAH concentration} \]
\[ C_{in} \times (ml/min/g \text{ wet weight of kidney}) = \text{clearance of inulin} \]

Results were represented as means±S.E. Statistical significance was assessed by Student’s t-test, P values less than 0.05 being considered significant.

Chemicals: 6-Aminonicotinamide was purchased from Sigma Chemicals Co. (St. Louis, U.S.A.), sodium pentobarbital (Nembutal) from Abbott Laboratories (Illinois, U.S.A.), inulin from Nakarai Chemicals, Ltd. (Kyoto, Japan), ATP Test-Combination from Boehringer Mannheim (Mannheim, West Germany), and para-
Results

The measurements of ATP and 6-PG contents in the kidney were carried out at intervals of 2 hours after administration of 6-AN.

Figure 1 shows the time-dependent changes of 6-PG content in the kidney. After the administration of 6-AN, the 6-PG content rapidly increased and reached a plateau at the fourth hour. This state was maintained from the fourth to the eighth hour, after which it decreased.

Figure 2 shows the time-dependent changes of ATP content in the kidney. The ATP content in the kidney was normally kept up to the sixth hour after the administration, after which it decreased showing statistical significancies.

These results revealed that the pentose phosphate pathway and the ATP formation in the kidney were inhibited later than the second hour and later than the eighth hour, respectively, by the administration of 6-AN.

Since the results of Fig. 1 indicated that the inhibition of the pentose phosphate pathway caused by 6-AN was maintained with a steady state from the fourth to the eighth hour after the administration, the following studies for the renal functions and hemodynamics were started at 6 hours after the administration of 6-AN.

The results of renal hemodynamics and functions in the control and in the 6-AN group before loading the rat with PAH were respectively as follows: glomerular filtration rate, 1.38±0.03 and 1.27±0.07 ml/min/g wet weight of kidney; renal plasma flow, 4.68±0.24 and 4.89±0.59 ml/min/g wet weight of kidney; renal blood flow, 8.41±1.04 and 8.62±1.06 ml/min/g wet weight of kidney; filtration fraction, 29.5±1.0 and 29.1±4.8%, with 6 experiments performed for each, in both groups. The values in the 6-AN group showed no significant differences in comparison to those in the control. Thus, these results denoted that the renal hemodynamics and functions were normal in the two groups before the PAH loading.

Next, the experiment to investigate the inhibitory effect of 6-AN on the renal transport of PAH was carried out. As described in Materials and Methods, the rat was loaded with 0.5% PAH solution from 6 hours after the administration of 6-AN.

Figure 3 shows the time-dependent changes of plasma concentrations of electrolytes and PAH before and during the loading of PAH. When compared to the values of the control at each time point, the plasma potassium in the 6-AN group showed no significant differences throughout the whole process, while the plasma sodium in the 6-AN group showed a higher tendency during the periods of 0–10, 20–30 and 40–50 min after the start of PAH loading. The PAH levels in plasma in the two groups
increased gradually in accordance with the infusion time elapsed, and there were no significant differences at each time point between the two.

Figure 4 shows the time-dependent changes of the urinary volume and urinary excretions of electrolytes and PAH before and during the loading with PAH. When compared at each time point, the urinary volume and urinary excretions of sodium and potassium were significantly increased during the PAH loading.

Figure 3. Time-dependent changes in plasma concentrations of sodium, potassium and PAH caused by PAH loading after administration of 6-AN. Vertical bars represent the mean±S.E. The number of experiments was 6 in each period. The values corresponding to each time point were statistically analyzed; asterisks represent the significant differences. The PAH loading was started at 0 min.

Fig. 4. Time-dependent changes in urinary volume and urinary excretions of sodium, potassium and PAH caused by PAH loading after administration of 6-AN. Explanations are as represented in Fig. 3.
potassium in the 6-AN group were significantly lower than those in the control at the following time points: at the periods of 15–20 and 25–45 min in the urinary volume, later than 30 min in the sodium, and throughout the whole process in the potassium. The urinary excretion of PAH in the two groups increased in accordance with the time elapsed; and in the 6-AN group, it showed a significantly lower tendency in comparison to that in the control at later than 10 min, excluding the periods of 30–40 and 45–50 min.

Figure 5 shows the time-dependent changes of glomerular filtration rate in the two groups before and during the PAH loading. There were no significant differences between the two groups at each time point: the glomerular filtration rate was kept at a normal level in these two groups in spite of the PAH loading.

Table 1 shows the values of renal tubular secretion maximum for PAH (T_{mPAH}) in the two groups. The value in the 6-AN group was found to be significantly depressed in comparison to that in the control.

Table 1. Values of renal tubular secretion maximum for PAH in the control and the 6-AN group

<table>
<thead>
<tr>
<th>Renal tubular secretion maximum for PAH (T_{mPAH}) (mg/min/g wet weight of kidney)</th>
<th>Control (N=6)</th>
<th>6-AN (N=6)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.153±0.056</td>
<td>0.872±0.023</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

The values represent the means±S.E. N denotes the number of experiments.

Discussion

This work was carried out using 6-AN, a typically potent inhibitor of the pentose phosphate pathway, with the aim of investigating changes of renal PAH secretion capacity under the inhibition of the renal pentose phosphate pathway caused by 6-AN.

In this study, 6-AN was intraperitoneally administered to the rat in a dose of 75 mg/kg body weight. This dose was higher in comparison to the reports published hitherto: 6 mg/kg (11), 35 mg/kg (12–14), 50–55 mg/kg (15, 16), and 35–75 mg/kg body weight (17, 18). To investigate the renal transport of PAH under the inhibition of the pentose phosphate pathway in the kidney, constant maintenance of this inhibition is indispensable for clearance studies during the PAH infusion because the studies for measuring PAH clearance, including the preparation for ureteral catheterization, needed a relatively long time of about 2 hours to complete. Providing that the increased 6-PG is a marker for the inhibition of the pentose phosphate pathway caused by 6-AN, this increased 6-PG in the kidney was confirmed to be maintained at a plateau level from the fourth to the eighth hour after the administration of 6-AN in a dose of 75 mg/kg body weight (Fig. 1). During this steady state, the renal tubular secretion maximum for PAH was studied.

According to Herken (2), the administration of 6-AN was reported to increase renal excretions of sodium, potassium, glucose and water in the unanesthetized rat. In contrast, in the present study, the urinary excretions of water and sodium in the 6-AN group were kept at the level of the control before the PAH loading, whereas those in the 6-AN group were significantly lower than in the control at approximately 30 min after the
start of the PAH loading (Fig. 4). Also, the urinary excretion of potassium in the 6-AN group was significantly lower than in the control before and during the PAH loading (Fig. 4). These findings in this study were opposite to those of the above reports (2), as if the 6-AN administration accelerated the renal tubular reabsorptions of water, sodium and potassium. In considering the causes for such opposite findings, the difference in the employed method might be one of the possible causes: the use of unanesthetized rats without saline infusion in the report of Herken (2) and the use of anesthetized rat with saline infusion in this study. If the 6-AN administration caused a decrease of the glomerular filtration rate, the difference in findings could be in part explained; The glomerular filtration rates in both groups were, however, kept at a normal level throughout the PAH loading (Fig. 5). Low level of PAH infusion was reported to increase the renal reabsorption of sodium (19, 20), and thus the co-transports of electrolytes with PAH should be discussed here. However, this study had a methodological limitation that the urinary fluid was investigated as the final urine but not as the tubular fluid at the nephron level. Therefore, it is a little unreasonable to further discuss these contrary findings, and these problems remain to be investigated using more specialized methods such as the micropuncture technique and so on.

NAD and NADP, which are coenzymes that are indispensable for glucose metabolic systems including the pentose phosphate pathway, have been considered to be partially substituted to 6-aminoNAD and 6-aminoNADP in the cells after the administration of 6-AN. (2) The renal 6-PG content in this study, in fact, was reconfirmed to be increased more than 100 times that of the control (Fig. 1), and this increased 6-PG possibly suggested an acceleration of glucose-6-phosphate dehydrogenase and/or an inhibition of 6-phosphogluconate dehydrogenase. According to the reports published hitherto (11, 13, 18, 21), 6-phosphogluconate dehydrogenase in the pentose phosphate pathway was inhibited in the kidney by administration of 6-AN. In addition, increased 6-PG was reported to inhibit phosphoglucoisomerase in the Embden-Meyerhof pathway (13, 21). Consequently, the glycolytic system was inhibited as a whole. In addition, Bruchhausen and Herken (22) and others (12, 15, 16) reported that 6-AN decreased the insulin-stimulated glucose uptake in cells and that it also decreased epinephrine content in adrenal glands, thus causing hyperglycemia and glucosuria. Furthermore, it was reported that 6-AN showed no hyperglycemic action in the adrenolecetomized rat (16). These reports possibly indicate that an increased discharge of epinephrine from the adrenal glands by 6-AN inhibits the glucose uptake and also that 6-AN inhibits the glycolysis in the proximal tubular cells.

From our results obtained in the PAH-loaded rats, it was found that the renal tubular secretion maximum for PAH was obviously depressed by 6-AN. PAH secretion is known to be energy-dependent, and its secretion capacity is considered to be affected by changes of intracellular level of high energy phosphate compounds, especially ATP (23–27). Accordingly, the decrease of secretion capacity for PAH could be in part explained by the decreased level of ATP, if the 6-AN decreased the ATP level by the inhibition of glycolysis. However, our finding showed no statistically significant change of the renal ATP content in the sixth hour after the 6-AN administration, in which the PAH loading study was carried out, whereas the ATP level was significantly decreased in the eighth and the tenth hour: When the renal ATP content at the starting point of the experiment was taken as 100%, its content in the sixth, eighth and tenth hour after the 6-AN administration was 87, 78 and 77%, respectively (Fig. 2). According to Weiner et al. (28), cyanide infusion (0.03 mg/kg body weight/min) into a renal artery in the dog caused a decrease of renal ATP content (58% of the control), while it did not significantly inhibit the renal PAH secretion. This suggests that the renal transport of PAH was not very sensitive to small changes in renal ATP levels. Therefore, in our study, the decrease of renal tubular secretion capacity for PAH could not be considered to
relate to the decreased level of renal ATP, since the degree of the decreased level of renal ATP observed in this study was relatively smaller than that in the report by Weiner et al. (28). However, since it is not certain whether a species difference in the renal secretion of PAH in relation to the renal ATP level exists between the dog and the rat, this needs further investigation.

PAH has been known to be transported transcellularly by an active step localized at the basolateral membrane and by diffusion across the luminal membrane in the proximal tubule, especially in the pars recta (23, 29–31). According to Cross and Taggart (31), the intracellular accumulation of PAH is enhanced by acetate, pyruvate, and lactate, while it is suppressed by short-chain fatty acids like hexanoate and octanoate and by dicarboxylic acids such as alpha-ketoglutarate, fumarate and succinate. By the administration of 6-AN, levels of the above and other substrates in both intracellular and interstitial fluid were considered to be changed in part, especially 6-PG (13). As a consequence, of course, when the cells in the proximal tubule are exposed to the interstitial fluid whose substrate components are extremely changed or when the intracellular components of the renal proximal tubular cells are largely changed, the renal transport capacity for PAH might be influenced as represented in the above report of Cross and Taggart (31). Although the effect of 6-PG on the intracellular PAH accumulation has not been reported, it is possible that changed intracellular and interstitial levels of 6-PG suppresses the renal transport capacity for PAH. However, this is only a speculation that needs further investigation.

In any case, an emphasis should be placed on the findings that the renal PAH secretion capacity was decreased in the renal glycolytic disturbance produced by 6-AN, and further investigations at the nephron level, including micropuncture studies and studies of single isolated nephron segments with exact measurements of enzymes and substrates, are needed for elucidating the mechanism(s) on the ATP-related transport systems in the inhibition of the glycolytic system.

Acknowledgement: This report was partially supported by grants from the special research foundation of Higashi-Nippon-Gakuen University (Grant No. 83PA-5 and 84PB-3).

References
7 Horecker, B.L. and Smyrniotis, P.Z.: Phosphogluconic acid dehydrogenase from yeast. J. Biol. Chem. 193, 371–381 (1951)
12 Ammon, H.P.T. and Steinke, J.: 6-Aminonicotinodi-
namide (6-AN) as a diabetogenic agent in vitro and in vivo studies in the rat. Diabetes 21, 143–148 (1972)


19 Vogel, G. and Kroger, W.: Die Bedeutung des Transports, der Konzentration und der Darbie-


