INHIBITION OF GLUCOSE REABSORPTION INDUCED BY 6-AMINONICOTINAMIDE IN THE RAT KIDNEY

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Abstract—The relationship between natriuresis and glucosuria produced by administration of 6-aminonicotinamide (6-AN), was investigated in the rat. After intraperitoneal administration of 6-AN (75 mg/kg), urine was collected at intervals of 2 hours using a metabolic cage for assays. Sodium and glucose were excreted maximally into the urine at 2 to 4 and at 4 to 6 hours, respectively, after the administration of 6-AN, with a time delay being recognized between sodium and glucose in the peaks of their urinary excretions. This dissociation of the patterns on the urinary excretion between sodium and glucose led to the following conclusion that the natridiuresis induced by 6-AN was not mainly ascribed to the osmotic diuresis for glucose, but to the direct effect on the sodium transport in the kidney. Furthermore, additional experiments were carried out by loading animals with glucose after administration of 6-AN. The tolerance for glucose in the body was clearly depressed in rats in the 6-AN group, while no significant difference in the renal threshold concentration for glucose was shown in either group. The renal tubular transport maximum for glucose was also depressed in the 6-AN group. It is, accordingly, speculated that the glucosuria induced by 6-AN was not only due to the hyperglycemia, but also due to the decreased capacity of the renal tubular reabsorption for glucose.

Key words : 6-aminonicotinamide, glucosuria, natriuresis, renal tubular transport maximum for glucose, rat.

INTRODUCTION

6-Aminonicotinamide (6-AN) is known to be a 6-amino derivative of nicotinamide. Coper and Neubert (1964) reported that this agent competitively inhibited those oxido-
reductases which were dependent on nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).

It is, furthermore, known that 6-AN exhibits a strongly toxic influence on various organs, producing such typical toxic symptoms as spastic paralysis (Herken et al., 1969; Herken and Lange, 1969), embryotoxic and teratogenic action (Chamberlain and Goldyne, 1970), inhibition of insulin-stimulated glucose transport into adipose tissue, hyperglycemia and glucosuria (Lange et al., 1972; Keller et al., 1972; Ammon and Steinke, 1972; Ammon, 1975), inhibition of renal transport of sodium (Herken, 1968) and para- amino hippuric acid (Sudo et al., 1984).

To elucidate the interrelated mechanism(s) of the above natridiuresis and glucosuria induced by 6-AN, the authors endeavored to determine whether this glucosuria was due to the hyperglycemia caused by the disturbance of glucose metabolism in the body, or to the decrease of glucose reabsorption in the kidney. Thus, basically being concerned with the study of metabolic disturbances of electrolytes and glucose in the kidney by administration of 6-AN, we also investigated the time-dependent relation between natridiuresis and glucosuria.

**MATERIALS AND METHODS**

In the first series of experiment, the time course changes in plasma and urine levels of electrolytes and glucose by administration of 6-AN were investigated.

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 intraperitoneally administered 6-aminonicotinamide (6-AN) (Sigma, U.S.A.) in saline (75 mg/kg body weight), and the other, namely the control group, being administered an equivalent volume of saline. Given free access to water and diet, each rat was kept in a metabolic cage, with urine being collected every 2 hours. Blood was collected from the abdominal aorta at the end of this experiment. Glucose in urine and plasma was assayed by the method of Schmidt (1961), using a spectrophotometer HITACHI-320 (Hitachi Co., Tokyo, Japan). Sodium and potassium in plasma and urine were measured using a flamephotometer FLAME-30C (JASCO Medical Instruments Inc., Tokyo, Japan).

In the second series of experiment, the renal clearance and tubular transport maximum for glucose in the kidney were investigated. This operation was begun at 4 hours after administration of 6-AN, since it was recognized in the above experiment that the urinary excretion of glucose reached the maximal level at 4 to 6 hours after administration of 6-AN.

The rats were anesthetized intraperitoneally with 50 mg/kg body weight of sodium pentobarbital, and were intubated for free respiration, after which a left femoral vein was catheterized with a polyethylene tube (PE-50). Throughout each experiment, saline containing 12 % inulin and 0.05 % p-aminohippurate (PAH) was injected at a rate of 2ml/kg body wt as a prime, followed by infusion of saline containing 0.08 % inulin and 0.05 % PAH at a rate of 1.5 ml/kg body wt/min : An equilibration period of at least 60
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min was allowed. The left ureter was approached retro-peritoneally, and was catheterized with a polyethylene tube (PE-10). (Sudo et al., 1983) Urine was collected every 5 min, with tail blood being taken at intervals of 10 min. For loading with glucose, the other solution containing glucose in a concentration of 5% in the above-mentioned infusion solution, was infused at the same rate. The infusion of glucose saline solution was begun at 6 hours after administration of 6-AN, as mentioned in the section RESULTS.

Glucose, sodium and potassium were measured as represented above. Inulin in urine and plasma was analyzed by the anthrone method of Führ et al. (1955) PAH concentrations in urine and plasma were analyzed according to the method of Smith et al. (1945) Urinary excretions of electrolytes and glucose were calculated using the clearance of inulin.

According to the report of Burgen (1956), and McPhaul and Simonaitis (1968), the renal threshold concentration for glucose was obtained by extrapolation of the titration curve, plotting the plasma glucose concentration (abscissa) and the urinary glucose concentration corrected by the inulin clearance (ordinate), with the renal tubular transport maximum for glucose (TmG) being calculated from the following formula:

\[ Tm_G (mg/min/kidney) = P_G \times C_{in} - U_G \times V \]

\[ P_G (mg/ml) = \text{plasma glucose concentration} \]

\[ C_{in} (ml/min/kidney) = \text{clearance of inulin} \]

\[ U_G (mg/min) = \text{urinary glucose concentration} \]

\[ V (ml/min/kidney) = \text{urinary volume per min per kidney} \]

Results were represented in the mean ± S.E. Statistical significance was assessed by Student's t-test, P values less than 0.05 being considered significant.

RESULTS

In the first series of experiment, the time-dependent changes in plasma and urine levels of electrolytes and glucose by administration of 6-AN were investigated.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Plasma concentrations of sodium, potassium and glucose at 10 hours after administration of 6-aminonicotinamide in rats.</th>
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<tr>
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<td>Control (N=6)</td>
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<tr>
<td>Na</td>
<td>mEq/L</td>
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<tr>
<td>K</td>
<td>mEq/L</td>
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<tr>
<td>Glucose</td>
<td>mg/dl</td>
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Values are represented as the mean±S.E. Abbreviations, N: the number of experiments. N.S.: not significant.

Table 1 shows the plasma concentrations of sodium, potassium and glucose in rats at 10 hours after the intraperitoneal administration of 6-AN. As seen in Table 1,
sodium showed no change, while potassium significantly decreased (p<0.001), and glucose significantly increased (p<0.05) in the 6-AN-pretreated rats as compared to the control group.

Fig. 1 shows the time-dependent changes in urinary volume and urinary excretions of sodium, potassium and glucose induced by the administration of 6-AN. The urinary volume did not significantly increase during the first two-hour period after the administration of 6-AN, but increased rapidly during the second two-hour period to attain its maximum (p<0.001). Thereafter it showed a gradual decrease, but was still higher than the control even during the fifth two-hour period. The urinary excretion of sodium showed the same tendency as the urinary volume, increasing maximally (p<0.001) during the second two-hour period after the administration, then gradually decreasing. No significant difference, however, was found in the urinary excretion of potassium between the two groups. The urinary excretion of glucose, differing from sodium, showed little increase during the second two-hour period after administration of 6-AN, but showed a rapid and significant increase (p<0.001) during the third two-hour period after the administration, following by a gradual decrease, although it remained higher than the control during the fifth two-hour period.
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From the above results, it was found that urinary volume ran parallel with the urinary excretion of sodium, reaching a maximum during the second two-hour period after the administration of 6-AN, while the urinary excretion of glucose reached its maximum during the third two-hour period after the administration, thus showing a time delay of 2 hours in attaining the peak of glucosuria as compared with the peak of both urinary volume and urinary sodium excretion.

In the second series of experiment, the inhibitory effects of 6-AN on the renal clearance and transport of glucose in the kidney were investigated. Since the results of the above experiment indicated that the 6-AN-induced glucosuria reached its peaks during the third two-hour period after the administration, it was suggested that some of the changes in the tubular reabsorption of glucose, if any, occurred from at least 4 to 6 hours after the administration. Accordingly, we started loading the rats with 5% glucose solution from 6 hours after the administration, in order to investigate the effect on the renal clearance and the renal transport of glucose.

Fig. 2 shows the results on plasma concentrations of electrolytes and glucose before and during the infusion of glucose solution. As to the plasma concentration, sodium and potassium showed no significant difference between the two groups throughout the whole process. Glucose levels in plasma increased gradually in accordance with the infusion time elapsed, the 6-AN group keeping markedly higher levels than the control group, and approached their maxima after the elapse of 30 min.

Fig. 3 shows the urinary volume, and the urinary fractional concentration of electrolytes and glucose before and during the infusion of glucose. The urinary volume and urinary fractional concentration of sodium and potassium in the 6-AN group, in general, showed no significant differences as compared to those of the control, although tendencies of increase in the urinary volume and of decrease in the fractional concentration of potassium in the 6-AN group were observed without significance, excluding the points of 50 to 60 min after the start of glucose infusion.

Results obtained in this experiment, differed from those obtained in the first series of experiment with concern to the natriuretic effect of 6-AN. This difference was thought to be due to being hidden by the infusion of a large amount of saline prior to and during loading with glucose.

The urinary fractional concentration of glucose, on the other hand, increased more rapidly and more remarkably in the 6-AN group as compared with that in the control, as seen in Fig. 3.

The calculated values of the renal threshold concentration for glucose (mg/dl) were as follows: 212.9 ± 10.0 in the control (N = 12), and 239.1 ± 10.8 in the 6-AN group (N = 12), with no significant difference being recognized between both groups.

The renal tubular transport maximum for glucose (TmC) (mg/min/kidney) in both groups was calculated by the formula mentioned in MATERIALS AND METHODS: 2.843 ± 0.199 in the control (N = 12), and 1.777 ± 0.175 in the 6-AN group (N = 12). The TmC in the 6-AN group was found to be significantly depressed in comparison with that in the control group (p < 0.01).
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Fig. 2. Changes in plasma concentrations of sodium, potassium and glucose produced by infusion of glucose after administration of 6-aminonicotinamide. Explanations are as represented in Fig. 1.

Fig. 3. Changes in urinary fractional concentrations of sodium, potassium and glucose and of urinary volume produced by infusion of glucose after administration of 6-aminonicotinamide. Explanations are as represented in Fig. 1

DISCUSSION

It has been considered that 6-aminoNADP, which is produced in the cells by administration of 6-AN, is partially substituted for NADP as a coenzyme indispensible to the glucose metabolic system including the pentose phosphate pathway. (Herken et al., 1975; Keller et al., 1972; Lange et al., 1970) According to their reports, 6-phospho-
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gluconate dehydrogenase in the pentose phosphate pathway was inhibited in the kidney by administration of 6-AN. In addition, the increased 6-phosphogluconate inhibited phosphoglucone isomerase in the Embden-Meyerhof pathway. Consequently the glycolytic system was inhibited as a whole. (Fig. 4)

Fig. 4. A schema of glycolysis.
(1) Inhibition of 6-phosphogluconate dehydrogenase by 6-aminoNADP.
(2) Inhibition of phosphoglucone isomerase by 6-phosphogluconate.

In our previous report (Sudo et al., 1984), when the rat was treated in the same way as in this study, it was found that the renal 6-phosphogluconate content rapidly increased, and reached a plateau at the fourth hour, with this level being maintained up to the eighth hour after the administration of 6-AN: its content increased to about 100 times that of the control. In contrast, the renal ATP content showed no statistically significant changes up to the sixth hour, while the ATP level was significantly decreased in the eighth and the tenth hour: when the renal ATP content in the starting point of experiment was 100 %, its content in the sixth, eighth and tenth hour after the 6-AN administration was 87, 78 and 77 %, respectively.

A main finding in the present study was that the renal tubular reabsorption capacity for glucose was depressed by the 6-AN administration. The renal active transport systems for glucose, para-amino hippuric acid and electrolytes are known to be energy-dependent, and the capacities are considered to be affected by changes of intracellular level of high energy phosphate compounds, especially ATP. (Dantzler, 1974; Spencer et al., 1979; Ulrich, 1979; Weiner et al., 1971) Accordingly, the decrease of reabsorption capacity for glucose could be in part explained by the decreased level of ATP in the kidney, if the 6-AN decreased the renal ATP level by the inhibition of glycolysis. However, the statistically significant decrease in the renal ATP level was not recognized in the sixth hour after the 6-AN administration, in which the glucose loading study was carried out. This suggests that such slight changes in the renal ATP level might affect the above active transport system for glucose in the kidney, although this is only a speculation that needs a further investigation.

In this study, the increase in plasma concentration of glucose was observed, and this increase was considered to be due to the disturbance of glucose metabolism in the body as represented above. Moreover, from the results obtained in glucose-loaded rats, it was observed that the tolerance for glucose was obviously depressed in the body by 6-
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AN, with this finding also being considered to relate the above disturbance of the glucose metabolism.

According to Amnon and Steinke (1972), Bruchhausen and Herken (1966), Schacht et al. (1966) and Schultz et al. (1966), it has been reported that 6-AN decreased the insulin-stimulated glucose uptake in the cells, and that it also decreased the epinephrine content in the adrenal glands. Furthermore, it was reported that 6-AN showed no hyperglycemic action in the adrenolecetomized rat. (Schultz et al., 1966) These reports possibly indicate that the hyperglycemic action of 6-AN is mediated by an increased discharge of epinephrine from the adrenal glands. As to the decrease in the plasma level of potassium observed in the 6-AN-treated rats, this is speculated to be a phenomenon related to the disturbance of the insulin-stimulated transport system as well as the increased discharge of epinephrine from the adrenal glands. In this point, further investigations are needed.

In this study, a further finding was that an obvious time delay existed in the increase of the urinary excretion between sodium and glucose by the administration of 6-AN: The increase in the urinary excretion of sodium characteristically preceded that of glucose. The authors believe that the above results are findings that should raise doubts as to the correlation between natriuresis and glucosuria caused by 6-AN. At least, it is certain that the natriuresis may not be or is not directly due to the osmotic diuresis induced by an increased concentration of glucose filtrated at the glomerulus.

As shown in Table 1, the plasma level of glucose at 10 hours after the administration of 6-AN, was 344.7 ± 23.0 mg/dl (N = 6). This level was beyond the value (239.1 ± 10.8 mg/dl) of renal threshold concentration for glucose in the 6-AN. Therefore, this hyperglycemia produced by 6-AN, in part, could be the first factor to increase the urinary excretion of glucose. This supposition had also been supported by the fact that 6-AN-treated rats showed no change in the renal threshold of glucose, notwithstanding the fact that the glucose concentration was markedly increased in urine in parallel with the increase in plasma level. It was demonstrated in our experiments, however, that the renal tubular transport maximum for glucose was depressed by 6-AN. The evidence appears to indicate that the inhibition of the tubular reabsorption is the second factor involved in the glucosuria induced by 6-AN.

In addition to the above-mentioned, our consideration concerning the dissociations of the patterns on urinary excretion between sodium and glucose is as follows. In the kidney, an osmotic gradient is formed from the cortex (300 mOsm) to the medullo-papilla (1200 mOsm) by the counter current system. (Berliner, 1976; Burg and Green, 1973; Rocha and Kokko, 1973) Thus, the tubular fluid and plasma in the medullo-papilla were possibly concentrated more than 4 times in comparison to those in the cortex. As a consequence of course, the Henle's loop and the collecting tubule existed in the medullo-papilla were exposed to high concentration of 6-AN, and these segments in the medullo-papilla could be affected faster than the cortical segments. The sodium filtered through the glomerular basement membrane, which is reabsorbed actively from the proximal tubule to the collecting tubule by the active transport which needs the
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energy derived from the glycolytic system and so on, could not be first reabsorbed because of the preceding inhibition of glycolysis in the medullo-papilla. Thereafter, the inhibition of glycolysis in the cortex is followed by that in the medullo-papilla. Thus, the glucose and sodium could not be in part reabsorbed actively in the proximal tubule.

From the above-mentioned, the following conclusions could be led that the natriuresis induced by 6-AN was not mainly caused by the osmotic diuresis for the increased glucose, and that the glucosuria induced by 6-AN was not only due to the hyperglycemia from the decreased capacity of tolerance for glucose in the body, but also to the decrease of the renal tubular transport maximum for glucose (Tm).}

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