Urinary constituents and renal function in rats administered with adenine

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Key words: adenine, urinary constituent, renal function, rat

Abstract

In adenine-administered rats, urine volume increased gradually with the progress of renal dysfunction to polyuria, but the amounts of urea, creatinine (Cr), guanidinosuccinic acid (GSA) and guanidinoacetic acid (GAA) excreted into the urine showed a significant decrease throughout the experimental period. In contrast, excretion of methylguanidine (MG) was markedly increased. The glomerular filtration rate (GFR), renal plasma flow (RPF) and renal blood flow (RBF) decreased progressively as the renal impairment increased due to prolonged administration of adenine.

Introduction

In chronic renal insufficiency, dysfunction of several processes in the living body, such as regulation of the body fluid composition, excretion, metabolism and endocrine secretion, can be observed. Finally, a uremic syndrome state is reached [1]. The basic nature of such uremia, however, remains unclarified in many respects and elucidation of its pathological physiology is therefore expected to provide some further insight into renal insufficiency. For this purpose, experiments on animals may yield useful information.

Conventionally, lateral renal resection and renal enucleation (the Platt method) are widely employed in studies on chronic renal insufficiency [2]. However, as an alternative approach, the present authors developed a simple method in which adenine is orally administered to rats. With lapse of time following the adenine administration, renal insufficiency develops accompanied by azotemia (elevated BUN, Cr), accumulation of uremic toxins such as methylguanidine (MG) and guanidinosuccinic acid (GSA), disordered electrolyte metabolism (Ca, P), a poor state of nutrition, abnormal amino acid patterns in the blood and urine, elevated blood pressure and disordered hormone secretion [3-9]. The biochemical findings in the adenine-fed rats bear a close resemblance to the metabolic abnormalities present in human renal failure. Pathological studies of the kidneys in such animals have revealed lesions of the proximal tubules, a proportion of the distal tubules and the glomeruli. Contracted kidneys have been found in rats with severe symptoms [6, 7]. Accordingly, the feeding of excessive adenine to rats is considered to provide an excellent model of chronic renal insufficiency.

In the present study, determinations of various urine constituents and renal clearance tests were carried out in an attempt to clarify the pathology of this disease, and new findings were obtained.

Materials and Methods

Animals and diet

Male Wistar rats (body weight, ca. 150g) were used for the experiments and were kept in metabolic cages at a temperature of 23 ± 1°C with a 12-hr dark-light cycle. Animals with renal insufficiency were prepared by feeding them on an 18% casein diet containing 0.75% adenine (dosage of adenine, ca. 350-400 mg/kg...
body weight), as in the previous studies [3-9]. The control animals were fed on an 18% casein diet only.

Analyses

During the adenine feeding period, urine collection was carried out every 2 days to determine the urinary excretion levels of urea, Cr and guanidino compounds. Urea was determined by the method of Archibald [10], and Cr by the Folin-Wu method [11]. Determinations of MG, GSA and guanidinoacetic acid (GAA) were performed using a Japan Spectroscopic liquid chromatograph employing a step-gradient system. A fluorescence spectrometer, model FP-210 (excitation 365 nm, emission 495 nm; Japan Spectroscopic Co., Tokyo, Japan) was used for detection of the substances on the column.

On the 6th, 12th, 18th, 24th and 30th day of the experimental diet, renal function tests were performed. The glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured by means of a renal clearance test employing a single intravenous administration of sodium thiosulfate or sodium para-aminohippurate, respectively, as the indicator [12, 13]. Thiosulfate and para-aminohippurate were determined by titrimentry and colorimetry, respectively. The renal blood flow (RBF) was calculated on the basis of RPF and the hematocrit value (Ht) using the equation shown below. Ht was determined with a hematocrit measurement apparatus, model KH-120A (Kubota Co., Ltd., Tokyo, Japan).

\[ RBF = \frac{RPF}{1 - Ht} \ (\text{ml/min}) \]

Statistics

The significance of differences between the normal and adenine-fed rats was evaluated by Student's t-test.

Results

Urine volume and urinary constituents: As shown in Fig. 1, the urine volume was maintained within the range of 18-28 ml/2 days in normal rats, whereas in the adenine-administered rats, it increased gradually with the progress of renal insufficiency, becoming approximately 3 times the normal value by the 18th day following administration. The level then decreased slightly and remained within the range of 40-50 ml/2 days. Urea excretion increased gradually in normal rats with growth, but in the adenine-administered rats, it dropped by 17-69% of the normal level. Similarly, the urinary excretion of Cr increased gradually with growth in normal rats, whereas in the experimental group, it was maintained within the range of 5.5-6.5 mg/2 days. This was a significant decrease by 40% of the normal value at the 16-18th day and persisted throughout the experimental period. As shown in Fig. 2, the amount of MG excreted into the urine accelerated markedly from the time of administration, the increase being essentially linear from the 16th to the 30th day. In contrast to the findings for MG, the urinary excretion of GAA dropped markedly from the time of administration, by 84% of the normal value, remaining at this level throughout the experimental period. The urinary excretion of GSA was depressed at the beginning and end of adenine administration, but was essentially the same as the normal value from the 10th to the 16th day. Over the experimental period as a whole, it was 37% less than the normal level.

GFR, RPF and RBF: As shown in Fig. 3, GFR in normal rats was 5.65 ± 0.27 ml/min/kg. This value decreased with time following adenine administration, dropping significantly by 51%, 61% and 80% of the level in normal rats on days 6, 12 and 18, respectively. After 24 days, it was 93% less than in normal rats and by the 30th day it was 96% less than the normal value. RPF was significantly decreased by the 12th day following administration, this decrease occurring later than that for GFR. The rate dropped to 11.32 ± 0.91 ml/min/kg from 20.83 ± 2.73 ml/min/kg, representing a significant decrease of 46% (p < 0.001). It had decreased by 75% after 18 days, by 92% after 24 days and by 97% of the normal value after 30 days. Ht was significantly decreased by the 12th day following administration. By 30 days, it had dropped by 36% of the normal level. RBF calculated from RPF and Ht showed a similar pattern of decrease to that of RPF, with a significant drop occurring by the 12th day. The decrease reached as much as 93%
Urine volume

(mg/2 days)

0 10 20 30 40 50

Urea

(mg/2 days)

0 200 400 600 800 1000 1200 1400

Cr

(mg/2 days)

0 2 4 6 8 10 12 14

Fig. 1. Urine volume, and urinary excretion levels of urea and creatinine. Cr, Creatinine.
■, Normal rats; □, adenine-administered rats. Values are means ± S.E. for 6 rats.
* Significantly different from the value for normal rats, p < 0.05; ** p < 0.001.
Fig. 2. Urinary excretion levels of guanidino compounds. MG, Methylguanidine; GSA, guanidinosuccinic acid; GAA, guanidinoacetic acid. ■, Normal rats; □, adenine-administered rats. Values are means ± S.E. for 6 rats. * Significantly different from the value for normal rats, p < 0.05; ** p < 0.001.
and 98% less than the normal values by the 24th and 30th days, respectively.

**Discussion**

In chronic renal insufficiency with abolishment of renal function, various clinical symptoms indicative of uremia become evident. In such cases, the decline in renal function results in decreased excretion from the kidney, causing retention in the body of uremic toxins which are considered to bring about uremia. The levels of some uremic toxins are increased in the excreted urine as well as in the blood with the progress of renal insufficiency, indicating possible acceleration of their production [1]. This abnormal increase reflects the pathology of renal insufficiency, particularly disordered nitrogen metabolism. Detailed examination of the increase is expected to help clarify the pathology of renal insufficiency.

Previously, the authors investigated the distribution of nitrogenous compounds in the serum of adenine-administered rats and found abnormally high levels of urea nitrogen, Cr, MG and GSA with lapse of time following administration [3, 5-7]. Furthermore, the level of MG was significantly increased in the cardiac muscle, liver, skeletal muscle, small intestine and kidney, while GSA was detected in the liver and kidney. The amounts of MG and GSA which accumulated in the body increased according to the number of days of adenine feeding. In contrast, the level of GAA became decreased in the kidney and serum [8].

In the present study, the urinary excretion levels of nitrogenous compounds were measured. Rats with renal insufficiency showed a decrease in the levels of excretion of urea, Cr, GSA and GAA into the urine. In particular, the urinary excretion of GAA was markedly decreased. The fall in production of GAA in the kidney appeared to be caused by a decrease in the level of precursors present due to reduction of the renal plasma flow and parenchymal injury in the kidney. It is thought that the amount of GAA excreted into the urine may reflect the state of metabolism in the kidney, thus clearly indicating lowered metabolic function in this organ, in adenine-administered rats. In addition, a remarkable fall in the urinary excretions of urea and Cr, which are dependent on glomerular filtration as the main route of excretion, was recognized concomitant with the decrease in GFR. These phenomena gradually became more marked as the period of adenine feeding progressed, resulting in a 55% decrease in urea and a 37% decrease in Cr over the whole experimental period. The excretion of GSA was also significantly decreased over the whole experimental period, although no change was observed from the 10th to the 16th day in comparison with the normal value. Stein et al. [14], Cohen [15] and
Koppel et al. [16] have already reported marked increases in the excretion of GSA into the urine with the progress of renal insufficiency. Although the differences between their findings and our present experimental results should be examined further, the protein intake of adenine-administered rats was confirmed to be approximately 65% of that in normal rats. It appears therefore that decreased protein intake could be a cause of GSA excretion (Cohen [15] reported an increase in the amount of GSA excreted into the urine as a result of ingestion of a high-protein diet). However, the results for GSA in adenine-administered rats indicated virtually no toxicity from the viewpoint of survival, in contrast to those for MG (data not shown).

On the other hand, the excretion of MG into the urine differed from that of other nitrogenous compounds, increasing to approximately 5.1 times the normal value over the whole experimental period. The level increased markedly with time following adenine administration. As reported previously [8], the content of MG in various organs was increased. On the basis of these findings, it is suspected that the production of MG itself increased. As Stein et al. [17] stated in their report, residual renal function in renal insufficiency may be significant. Our experimental findings suggest that MG is excreted into the urine by tubular secretion. It is worthy of note that GFR was decreased significantly 6 days after adenine administration, and that after 12 days, RPF and RBF fell significantly. FF (GFR/RPF) was determined to be 0.25 in normal rats, while significantly lower values of 0.15 after 6 days and 0.20 after 12 days were obtained. However, the values of 0.22 after 18 days and 0.29 after 24 days showed no significant difference from those in normal rats. On the other hand, this ratio was significantly increased to 0.37 after 30 days. In general, FF decreases in cases of glomerular disease, is mostly normal in tubular or interstitial diseases, and tends to increase in vascular renal diseases [18]. Light microscopy of renal tissue from adenine-administered rats indicated degeneration of the proximal tubular epithelium during the initial period; and then with lapse of time following administration, dilation of the distal tubules and the collecting ducts, fibrosis of the interstitium, and cellular infiltration were noted. A decrease in the number of glomeruli and hypertrophy of Bowman's capsules were also evident [6, 7] but did not always parallel the results for the renal function tests conducted in the present experiments. The cause-effect relationship between the data of renal function tests and kidney morphology should be examined in greater detail, although it is worthy of note that soon after adenine administration, precipitation of 2, 8-dihydroxyadenine, an adenine oxide, was observed mainly in the proximal tubules [19]. This substance accumulated in large amounts with time following administration. Obstruction of the proximal tubules by precipitation of this substance may possibly cause oppression and atrophy, or result in their dropping out from the local surrounding tissues. A decrease in GFR would be the first apparent result of such a process.

References

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