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Analysis of Differences in Urine Concentration Function and Erythrocyte Membrane Permeability among Different Animal Species

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Abstract: Through comparative studies on renal structure and urine concentration function, this research aims to observe and analyze the differential mechanisms among different animal species. Using rats as the research subjects and employing a high-protein feeding method, the study explores the mechanism of urea's effect on urine concentration function from multiple levels, including gene expression regulation and protein levels. Erythrocyte membrane permeability tests are conducted to compare the degree of differences among various animal species. This study utilized experimental techniques such as RT-PCR, Western-blot, Stopped-flow, and immunohistochemistry to investigate the urine concentration mechanism at the cellular, tissue, and whole-organism levels, laying a foundation for further screening and preparing animal models for studying the urine concentration mechanism.

Keywords: Urine concentrating function; Erythrocyte membrane permeability; Different species; Differential analysis

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1. Introduction

The kidneys are the largest excretory organs in the human body, playing a crucial role in regulating water volume and the excretion of various components [1]. This study intends to employ a variety of research methods, including comparative physiology and molecular biology, to comparatively analyze differences in renal physiology, body fluid and metabolic ion content, erythrocyte membrane permeability to water and urea, and renal urea channels. The aim is to explore the relationship between these factors and urine concentrating function, thereby further elucidating the mechanism of urine concentration in mammalian kidneys.

2. Materials and methods

2.1. Experimental materials and instruments

2.1.1. Materials

(1) Experimental animals

All animals used in this study were bred in-house, totaling six species. These include the following: rodents (mice, rabbits), herbivores (sheep, cattle), and carnivores (cats, dogs). The experimental animal grades were as follows: rabbits, dogs, and mice all meet the national standards for second-grade experimental animals, while the other experimental animals have undergone a two-week isolation observation period to confirm their health status and absence of diseases.

(2) Reagents

4% paraformaldehyde, disposable vacuum coagulation-promoting blood collection tubes, Sumianxin, urea detection kits, PBS buffer solution, urea, urea channel protein inhibitors, and other conventional reagents were all of analytical grade purity.

2.1.2. Instruments

Vernier calipers, electronic platform scales, precision electronic balances, pH meters, clean benches, refrigerators, fully automatic electrolyte analyzers, high-speed centrifuges, photometers, spectrometer reactors, blood analyzers, cryogenic centrifuges, clean benches, etc.

2.2. Methods

2.2.1. Comparison of structural characteristics of kidneys in animals of different species

Among the six experimental breeds, three animals were included in each group for a 30-day quarantine period. Daily monitoring of their food and water intake, physical condition, and excretions was conducted, while maintaining a constant temperature (20 °C) in the animal housing. After dissection, morphological observations were made on the organs of rats in each group. The kidneys were harvested, rinsed with TBS, dried, weighed, and longitudinally sectioned for counting. The kidneys of the experimental animals were measured using vernier calipers in the following order of parameters: absolute length, width, and thickness of the kidney were determined, then the kidney was bisected along its long axis with a double-edged blade along the median plane, and the thickness of each layer was directly measured on the cross-section using vernier calipers. Finally, tissue sections of the renal cortex and medulla from the rat kidneys were prepared, washed with TBS, longitudinally sectioned to 3 mm, and stored in liquid nitrogen; subsequently, different renal tissues were eluted with chloroform, fixed with formalin solution, and subjected to further immunohistochemical detection [2,3].

2.2.2. Comparison of ion and urea content in animal urine and serum ion content in animals

Before handling the experimental animals, they were fasted for 24 hours and deprived of water for 2 hours. Morning urine samples were collected from each mouse, with strict recording of their volume and state, and stored at 4 °C. Meanwhile, the concentrations of ions such as Na, K, Cl, and urea in the urine were measured. A 100 g sample from each part of the kidney was weighed, ground, dissolved in distilled water, and then centrifuged to determine the content of sodium, potassium, chlorine, other ions, and urea nitrogen. The content of ions such as Na, K, and Cl in rat urine, serum, and various renal tissues was detected using high-performance liquid chromatography [4,5].

2.2.3. Comparative study on the difference in permeability of animal erythrocyte membranes to water and urea

A blood analyzer was used to examine erythrocytes from different species, measuring their hematocrit, erythrocyte count, and erythrocyte volume to conveniently understand the hemorheological properties of erythrocytes. Centrifugation was performed at room temperature at 1000 r/min for 10 minutes to separate the erythrocytes, which were then washed three times with PBS and the blood discarded. Subsequently, using PBS buffer solution, the permeability of erythrocytes to urea and water was measured separately using a stopped-flow assay. Urea permeability assay: The prepared erythrocyte suspension (0.5% hematocrit) was mixed with 250 mM urea, and the dynamic changes in cell volume were measured at 530 nm with 90-degree light scattering. Each erythrocyte sample was repeated five times, and the light scattering time curve was calculated by computer to obtain the osmotic urea permeability (Purea). Water permeability assay: The freshly prepared erythrocyte suspension (0.5% hematocrit) was placed in a 250 mM sucrose solution, and the same procedure as above was followed, comparing it with the measurement method for water permeability (Pf) [6].

2.3. Statistical analysis

All collected numerical data were entered into SPSS 20.00 software for statistical analysis. Measurement data were recorded as mean and standard deviation.

3. Results and analysis

3.1. Comparison of structural characteristics of kidneys in different animal species

The study revealed significant differences in the relative thickness of the medulla among various species, with carnivorous animals exhibiting significantly thicker renal medullae compared to herbivores. Research on the proportion of different anatomical regions of the kidneys indicated that herbivores had the highest proportion of cortex, while rodents had the highest proportion of medulla (see **Table 1**).

Table 1. Comparison of kidney weight and structural characteristics among different animals

Species	Sheep	Cattle	Cat	Dog	Rabbit	Mouse
Body weight (kg)	36.52 ± 36.00	326.50 ± 58.00	2.368 ± 12.23	10.235 ± 13.25	1.668 ± 20.12	0.026 ± 0.35
Kidney weight (kg)	0.092 ± 2.54	1.054 ± 23.02	0.01 ± 0.35	0.05 ± 0.35	0.013 ± 0.23	0.00018 ± 0.12
Relative kidney weight (%)	0.252	0.322	0.422	0.489	0.779	0.692
Absolute cortex thickness (mm)	5.2 ± 0.3	5.7 ± 0.4	6.7 ± 0.3	8.4 ± 0.2	2.5 ± 0.6	1.0 ± 0.3
Absolute medulla thickness (mm)	7.0 ± 0.2	7.3 ± 0.4	8.8 ± 0.7	26.1 ± 0.5	8.3 ± 0.3	4.6 ± 0.2
Relative medulla thickness	0.57 ± 0.13	0.55 ± 0.05	0.68 ± 0.01	0.72 ± 0.08	0.74 ± 0.16	0.80 ± 0.12

3.2. Comparison of urinary ion and urea content in animals

The study demonstrated a strong correlation between sodium ion concentration in urine and diet, with higher sodium ion concentrations in food leading to increased sodium ion levels in urine. The results indicated that rodents had the highest urea nitrogen concentration, suggesting the strongest urine concentration capacity. Carnivorous animals, due to their long-term protein-rich diet, also exhibited higher overall BUN levels compared to rodents. Herbivores, on the other hand, maintained relatively low blood urea concentrations in their urine,

attributed to their lower protein intake over extended periods. The aforementioned studies indicate that due to long-term dietary habits and evolutionary characteristics, there are significant species-specific differences in the urine concentration capacity of kidneys among various species.

3.3. Comparison of serum ion content in animals

Through a comparative study of serum ion concentrations among various species, the results revealed the following: sodium (Na) ion concentrations ranged from 136 to 150 mmol/L, with no significant differences observed among species; potassium (K) ion concentrations ranged from 3.94 to 7.131 mmol/L, also showing no significant differences among species; and chloride (Cl) ion concentrations ranged from 102 to 130 mmol/L, with no significant differences among species as well.

3.4. Comparison of the effects of different protein intakes on urine concentration function

Research indicates that protein intake has a significant impact on the urine concentration function in rats, and the expression of aquaporin AQP2 and UT-A3 is also influenced by protein intake, confirming that urea generated from protein metabolism plays a significant role in regulating renal concentration capacity (refer **Figure 1**).

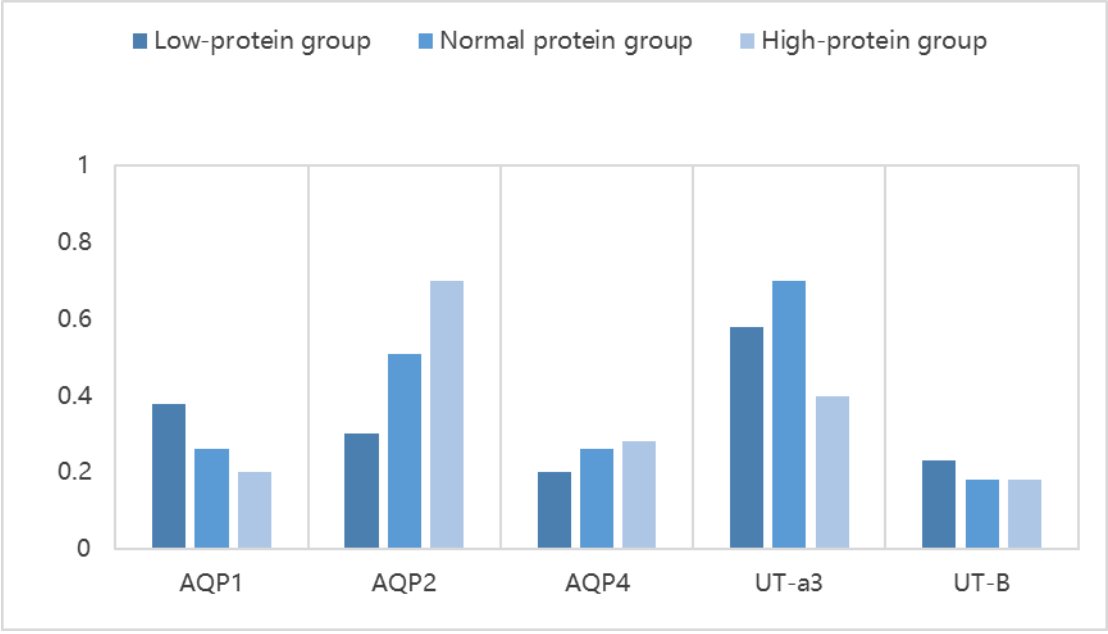

Figure 1. Quantitative analysis of urea transporter proteins and aquaporin proteins in rat kidneys with different protein intakes ($p < 0.05$).

3.5. Comparison of the permeability differences of water and urea in animal erythrocyte membranes

By comparing the differences in urea permeability of erythrocyte membranes among different species, this study discovered that there is species-specificity in the urea permeability of erythrocyte membranes, with the order being carnivores > rodents > herbivores; however, the water permeability of erythrocyte membranes is similar across different species.

4. Discussion

By comparing and observing the renal physiological structures of six species, measuring and calculating the absolute thickness of the renal cortex and medulla as well as their proportions in the kidneys of each species, this study confirmed that there are certain differences in renal tissues among different species both physiologically and anatomically. These differences provide a basis for further experimental research.

The experiment detected Na, K, Cl ions and urea N in the urine, serum, and various parts of the kidneys of six types of animals. It was concluded that there are no significant differences in serum sodium, potassium, and chloride ion contents among different species, but there are significant interspecies differences in blood urea nitrogen content. The variations in ion concentrations and urea concentrations in renal tissues are consistent with the differences in urine concentration abilities among different species [7].

By employing methods such as fluorescent quantitative RT-PCR, Western-blot, and immunohistochemistry, we systematically studied the effects of different protein diets on renal function in rats and revealed their regulatory mechanisms [8]. As the protein content in the feed increases, urine volume significantly increases. Different protein diets have no obvious effects on serum and urea nitrogen, but urea nitrogen in urine and urea clearance rate increase with the rise in protein concentration.

The stopped-flow light scattering method was employed to examine the permeability of erythrocyte membranes from six different animal species to urea, revealing significant interspecies differences [9]. However, the permeability to water was found to be similar across species, with no notable differences observed.

5. Conclusion

In summary, this study demonstrates that hyperuricemia induced by a long-term high-protein diet can lead to adaptive changes in renal structure and function. The elevated urea concentration resulting from a high-protein diet significantly affects urine concentrating ability and the expression of channel proteins related to urine concentration.

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Disclosure statement

The authors declare no conflict of interest.

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