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Relation between Natriuresis and Urinary Excretion of Hydrogen Peroxide

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Changes in the urinary hydrogen peroxide by exercise or salt load were studied in six healthy male volunteers. Exercise was performed by bicycle ergometer for 30 min at the intensity of 80% of the maximum heart rate predicted by age. Urinary excretion rate of hydrogen peroxide showed a tendency to increase in the salt load experiment, and to decrease by exercise. Correlation coefficient between urinary excretion rate of sodium and hydrogen peroxide one hour after the load was 0.797 ($0.1 > p > 0.05$) in the exercise experiment, 0.892 ($p < 0.05$) in the salt load experiment and 0.877 ($p < 0.001$) in both experiments. Correlation coefficient between area under the curve for sodium excretion and hydrogen peroxide excretion was also as high as 0.822 ($p < 0.05$) in the exercise experiment, 0.909 ($p < 0.05$) in the salt load experiment and 0.853 ($p < 0.001$) in both experiments. These results may suggest that urinary excretion rate of hydrogen peroxide is closely related to metabolism of electrolytes and fluid in the renal tubules.

Keywords: Hydrogen peroxide, sodium, urine, exercise

INTRODUCTION

A number of experimental studies provide strong evidence for an important role of reactive oxygen species, particularly hydrogen peroxide in the pathogenesis of renal injury.[1–8]

A highly sensitive radioactive method of peroxide determination in aqueous and semiaqueous media was developed by Varma.[9] The method is sensitive down to subnanomolar concentrations, and was applied to measure excretion of hydrogen peroxide in human urine.[10] Our previous study[11] showed that concentrations of hydrogen peroxide were inversely related to urine volume, and urinary excretion rate must be considered in pathophysiological studies. However, changes in urinary hydrogen peroxide according to physiological or clinical conditions have not been well studied.

In order to evaluate physiological characteristics of changes in urinary hydrogen peroxide excretion, we studied the relation between

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natriuresis and urinary hydrogen peroxide excretion in cases of exercise and salt load.

MATERIALS AND METHODS

Experimental Protocol

Six healthy male volunteers, ranging in age from 19 to 24 years old, in mean body weight from 58 to 73 kg and in body mass index from 20.1 to 24.2 participated in this study. None showed proteinuria. All subjects were informed of the procedures, and gave their consent for participation.

They voided their bladder and discarded that collection in the morning and then took 200 ml of water plus 400 kcal of a balanced nutrition food "Calorie Mate" (Ohtsuka Pharmaceal Co. Lt., Tokyo) consisting of protein 13.4 g, fat 8.8 g, sugar 66.8 g, salt 0.8 g and vitamins. Thereafter, 300, 200 and 50 ml of water were taken at 1, 2 and 3 h, respectively, and timed urine collections were obtained hourly for 4 h.

On other days, exercise and salt load were carried out at one hour. Exercise was performed by bicycle ergometer for 30 min at an intensity of 80% of the maximum heart rate predicted by age. Mean heart rate and work load during exercise were 174 ± 4/min and 160 ± 17 W, respectively. In salt load experiment, 6 g of salt was taken orally with 300 ml of water at 1 h.

Materials and Methods

All the chemicals used in this study are routinely available. [1-^{14}C]-alpha-ketoglutaric acid (disodium salt) was obtained from New England Nuclear Company, Boston, MA, USA.

After measuring volume, fresh urine sample was diluted 50 times with Tyrode buffer. Urinary concentration of hydrogen peroxide was measured by a modified method of Varma and Devamanoharan,[10] as previously reported.[11] The reaction mixture consisted of 250 µl of alpha-ketoglutarate reagent, 1250 µl of urine sample or standard hydrogen peroxide solution and 250 µl of Tyrode buffer. A reaction vial contained 250 µl of 0.2% catalase solution instead of Tyrode buffer and served as blank for each sample and standard. The hydrogen peroxide concentration of urine samples was determined from a standard curve. The hydrogen peroxide concentration in the undiluted urine sample was calculated by multiplying the result by the dilution factor. Urinary excretion rate of hydrogen peroxide was determined by multiplying concentration with volume of one hour urine sample.

Concentrations of sodium and creatinine in urine were measured by autoanalyzer CX-7 (Beckman, USA).

Area under the curve (AUC) for urinary excretion of sodium and hydrogen peroxide was calculated geometrically[12] for 3 h. Analysis of variance was carried out using Macintosh statistical package software Statview 4.0 (Abacus Concepts, USA) after logarithmic transformation of urinary excretion rate of hydrogen peroxide. Regression equations were obtained with a commercially available program (Cricket Graph) by Macintosh Classic personal computer (Apple Computer, Cupertino, CA). P values of < 0.05 were considered statistically significant.

RESULTS

In the control experiment, urine volume was increased to a peak of 221.3 ± 4.5 ml/h at 3 h. On the contrary, it was not changed by the exercise or salt load experiment as shown in Figure 1A. Urinary excretion rate of sodium was decreased by exercise from 9.7 mmol/h at 1 h to 5.7 mmol/h at 2 h. In the salt load experiment, it was increased gradually from 7.8 to 20.7 mmol/h (Figure 1B). The maximum value of urinary excretion rate of hydrogen peroxide in the salt load experiment was 12.6 ± 6.2 at 3 h. It showed a tendency to decrease in the exercise experiment to the minimum value of 6.9 ± 3.6 mmol/h at 2 h ($p = 0.058$) as shown in Figure 2. The minimum value in the

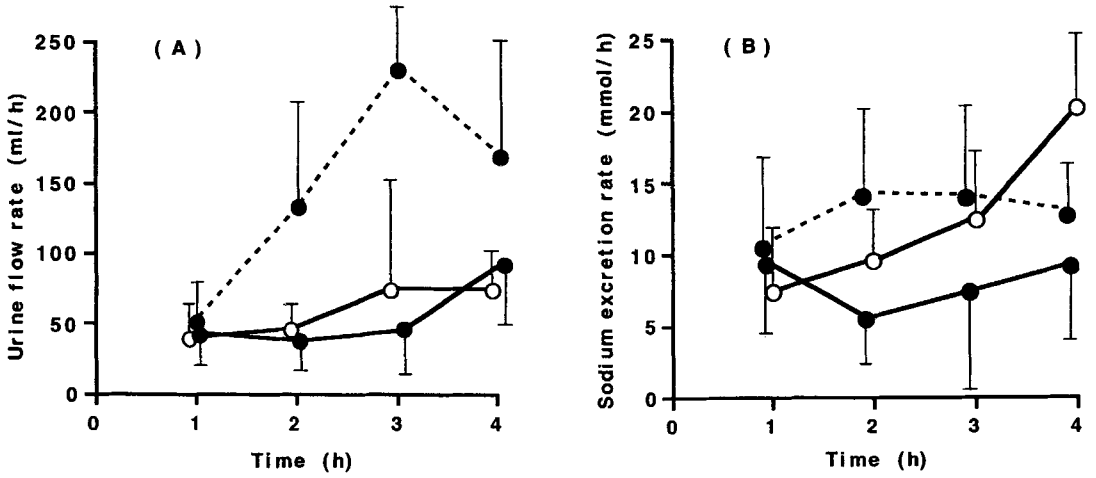

FIGURE 1 Changes in urine flow rate (A) and sodium excretion rate (B). •·····•; control experiment, o——o; salt load experiment, •——•; exercise experiment. $n = 6$; values are expressed as mean ± SD.

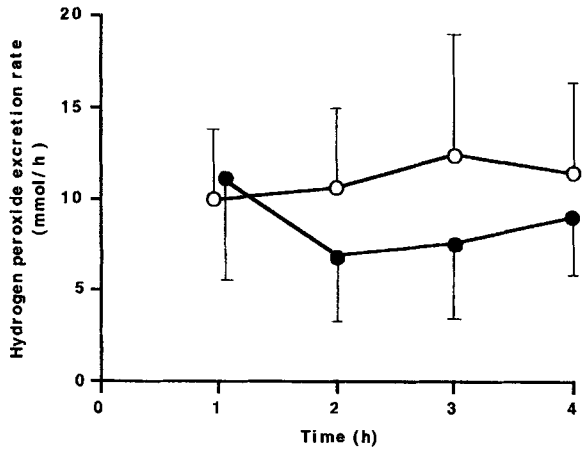

FIGURE 2 Changes in urinary excretion rate of hydrogen peroxide. o——o; salt load experiment, •——•; exercise experiment, $n = 6$; values are expressed as mean ± SD.

exercise experiment was about a half of the maximum value in the salt load experiment ($p = 0.105$).

The relation between urinary excretion rate of sodium and hydrogen peroxide is shown in Figure 3. Correlation coefficient for equation between both rates at 2 h was 0.797 ($0.1 > p > 0.05$) in the exercise experiment, 0.892 ($p < 0.05$) in the salt load experiment and 0.877 ($p < 0.001$) in both experiments. Correlation coefficient for equation between area under the curve for sodium and hydrogen peroxide excretion rate was also as high as 0.822 ($p < 0.05$) in the exercise experiment, 0.909 ($p < 0.05$) in the salt load experiment and 0.853 ($p < 0.001$) in both experiment as shown in Figure 3B.

Correlation coefficient for equation between urinary excretion rate of hydrogen peroxide and urine flow rate was also high. However, there was no significant relation between urinary excretion rates of hydrogen peroxide and creatinine (Table I).

DISCUSSION

We studied changes in urinary hydrogen peroxide by exercise or salt load for the first time, using a modified Varma and Devamanoharan's method.[10]

In the previous study,[11] we showed that urinary excretion rate of hydrogen peroxide is a proper function to be used in pathophysiological studies compared to concentration or ratio to creatinine. Mean excretion rate of hydrogen peroxide in this study was 10.0 ± 3.7 mmol/h before exercise and 11.3 ± 5.8 mmol/h before salt

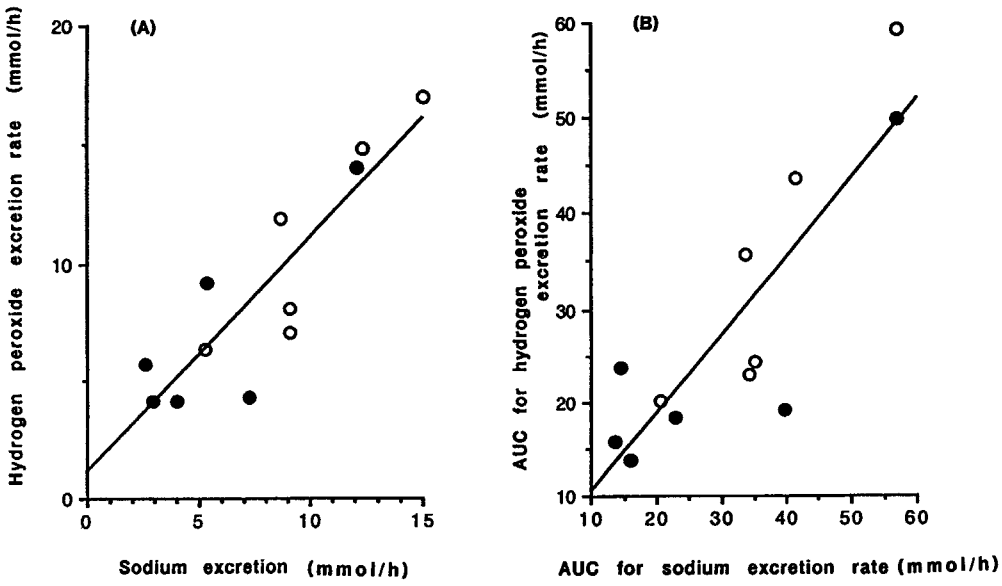

FIGURE 3 Relation between urinary excretion rate of sodium and hydrogen peroxide. (A) excretion rate at 2 h, regression equation $y = 1.14 + 0.995x$ ($r = 0.877$, $p < 0.001$). (B) area under the curve (AUC) for excretion rate, regression equation $y = 2.176 + 0.831x$ ($r = 0.853$, $p < 0.001$). $n = 6$; ○; salt load experiment, ● exercise experiment.

TABLE I Correlation coefficients for equations between urinary excretion rates of hydrogen peroxide and water, sodium, and creatinine

	Correlation coefficient		
	Urine volume	Sodium excretion	Creatinine excretion
Hydrogen peroxide excretion			
2 h	0.731**	0.877***	0.348*
AUC for 3 h	0.732**	0.853**	0.432*

AUC: area under the curve.
* $p > 0.1$, ** $p < 0.01$, *** $p < 0.001$.

load, which are consistent with values in the previous study.[11]

Mean excretion rate of hydrogen peroxide showed tendency to decrease by exercise to 61% of control level ($p = 0.18$), and to increase by salt load to 126% ($p = 0.773$). It was reported that exercise at heavy intensity decreases glomerular filtration rate moderately to 40%.[13–15] A salt load of 6 g may increase glomerular filtrate of sodium by several percent. The difference in the magnitude of changes in sodium load to renal tubules may contribute to the difference in the magnitude of changes in urinary excretion of hydrogen peroxide observed in the both experiments.

In this study, urinary excretion rate of hydrogen peroxide did not show significant correlation with that of creatinine, and showed highly significant relation between urinary sodium excretion rate and urine flow rate, especially between the former. These results may suggest that urinary excretion rate of hydrogen peroxide is related to electrolytes and fluid metabolism in the renal tubules.

Performance of dynamic exercise is associated with an antinatriuresis,[16] and it was reported that urinary sodium excretion and per cent filtered sodium excreted decreased significantly during heavy exercise. A part of this reduction

may be explained by the decrease in glomerular filtration rate. However, Castenfors *et al.*[16] reported that sodium excretion decreased markedly during exercise and was not related to a decrease in GFR. Exercise at intensity of 80% performed in this study may produce elevation in plasma concentration of catecholamines,[17,18] ANP,[13] aldosterone[13,17] and vasopressin,[13,17–19] renin activity,[13,19] and others.[18] Combining these factors with decrease in glomerular filtration rate, urine flow rate was supposed to be decreased, which was compensated by drinking 750 ml of water during the experiments in this study. These factors, especially increase in plasma aldosterone,[13,17] may contribute to reduction in urinary sodium excretion rate to about 60% levels of control value, which was observed in the exercise experiment. On the other hand, oral intake of salt may increase plasma concentrations of ANP, vasopressin, sodium load to renal tubules and urinary excretion rate of sodium, and decrease in plasma aldosterone in the salt load experiment.

Many factors contribute to changes in sodium excretion rate observed in the both experiments, and it is not clear yet which factors influence primarily on both of urinary excretion rate of hydrogen peroxide and sodium. Further studies are needed to elucidate mechanism of changes in urinary excretion rate of hydrogen peroxide by exercise and salt load.

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