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Thermal Stress Regulates Erythrocyte Polyol Metabolism

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熱ストレスによる赤血球ポリオール代謝の調整

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抄　録

糖尿病性合併症の成因の一つに，アルドース還元酵素（AR）活性の亢進に伴う細胞内ソルビトール濃度上昇に代表される，ポリオール代謝異常が挙げられている。温熱療法は種々の疾患のリハビリテーションに応用されているので，今回温熱ストレスの赤血球ポリオール代謝に与える影響を検討した。9名の健常男子大学生を日を変えて，3種類の水温で10分間水浸させ，末梢静脈血を採血した。赤血球のAR活性は42℃で1.09±0.19から1.50±0.25U／gHbへと増加し（$P<0.01$），39℃と25℃とではそれぞれ0.92±0.08から0.44±0.08U／gHb（$P<0.01$），1.08±0.14から0.54±0.09U／gHb（$P<0.05$）へと減少した。赤血球ソルビトール濃度は42℃にて32.7±5.8から43.7±8.8nmol／gHbへと上昇した（$P<0.05$）。赤血球をin vitroで温度を変えてインキュベーションすると，42℃では明らかにAR活性が上昇したが，39℃では変化がなく25℃で軽度上昇した。また赤血球を39℃でアドレナリンの存在下でインキュベーションすると，アドレナリンの濃度依存性にAR活性とソルビトール濃度が増加した。これらのことから，①熱ストレスは糖尿病性合併症を悪化させる可能性が考えられた。②AR活性の調節にアドレナリンが関与している可能性が考えられた。

Key words : Erythrocyte, Aldose reductase, Thermal stress, Water immersion, Epinephrine

INTRODUCTION

The polyol pathway is strongly related to diabetic complications such as neuropathy, retinopathy, cataract and nephropathy[1),2)]. It consists of two steps in which glucose is

first reduced to sorbitol by the enzyme aldose reductase (Aditol: NADP oxidoreductase, E.C.1.1.1.21(AR)) in an NADPH-dependent reaction. Then the sorbitol is converted to fructose by sorbitol dehydrogenase (Iditol dehydrogenase, E.C.1.1.1.14) in an NAD^+-dependent reaction. One of the mechanisms responsible for the complications is thought to be enhanced activity of AR[3], which leads to accumulation of sorbitol and fructose in the cells, resulting in osmotic imbalance, depletion of myoinositol, and cellular death[4)-6)]. This enzyme activity has been examined in various tissues and the activity in erythrocytes is quite well correlated with those from other tissues and with the extent of diabetic complications[2),7),8)].

Patients with peripheral circulatory disorder from diabetic neuropathy and angiopathy usually feel relieved from symptoms such as pain and numbness after bathing. However, it is unclear whether the water temperature is important or not ; moreover, no experiment has been done to investigate the influence of heat stress on the polyol pathway.

In the present study, to clarify the effect of heat stress on diabetic complications, we immersed men and also we incubated erythrocytes at 3 different water temperatures, 25℃, 39℃ and 42℃, and investigated the changes of erythrocyte polyol metabolism.

MATERIALS AND METHODS

Materials

DL-Glyceraldehyde was purchased from Wako Pure Chemical Industries. NADPH was from Sigma (St. Louis). All other reagents were of analytical grade.

Water immersion design

The effect of water temperature on the AR activity was investigated at 25℃, 39℃ and 42℃ in 9 normal male students (20.1±0.2years). Sorbitol concentration was measured in 7 out of 9 subjects after immersion at 42℃. After 5-10 min rest in a chair at room temperature (27℃), venous blood was drawn, and then the subjects were immersed in a long sitting position (sitting position with legs outstretched) in a water bath for 10 min. A second blood sample was taken immediately after the end of the immersion. Water immersion at different temperatures was carried out with at least a one-week interval between immersions. The Ethics Committee of the Hokkaido University School of Medicine approved this experiment and informed consent was obtained from all subjects.

In vitro study

To know the effect of epinephrine on erythrocyte sorbitol metabolism, cells were incubated with different concentrations of epinephrine (0-5.0 μmol/l) in a TES buffer(1% BSA, 8 mmol/l glucose, 62 mmol/l NaCl, 40 mmol/l sodium phosphate, 35 mmol/l TES, pH7.4) at 39℃ for 30 min. After the incubation, erythrocytes were washed three times with cold isotonic saline, then the enzyme activities and the sorbitol

concentrations were measured. Erythrocytes were also incubated in a TES buffer at three different temperatures, 25℃, 39℃ and 42℃, for 30 min. After washing three times with cold isotonic saline, AR activities were determined. No hemolysis was observed during incubation.

Assay methods

Venous blood collected in 1 mg of EDTA/ml was passed through a column of α-cellulose and microcrystalline cellulose (1:1, W/W)to remove platelets and leukocytes[9]. Erythrocytes were washed three times with cold isotonic saline and frozen at −70℃ and then thawed to effect hemolysis. The hemolysate was used for assays of AR and activity was determined with glyceraldehyde as a substrate. The assay system, as described previously[10] and briefly modified, contained 50 mmol/l potassium phosphate (pH 6.0), 5 mmol/l 2-mercaptoethanol, 1 mmol/l lithium sulfate, 0.1 mmol/l NADPH and diluted hemolysate with or without (as a blank) 10 mmol/l DL-glyceraldehyde in a total volume of 0.5 ml. The reaction was started by addition of the substrate. The decrease in absorbance at 340 nm was recorded at 37℃ for 20 min. Enzyme activity was defined as the number of micromoles of NADPH oxidized per minute at 37℃. Erythrocyte sorbitol content was determined according to the method of Bergmeyer *et al.*[11]. The hemoglobin (Hb) concentration of each hemolysate sample was measured by the cyanmethemoglobin method.

Statistical analysis

Wilcoxon test was used for the analysis of the study. A probability level of 0.05 or smaller was used to indicate statistical significance. Data are expressed as mean±SEM.

RESULTS

Effect of water immersion at different temperature

AR activity was increased from 1.09±0.19 to 1.50±0.25 U/gHb ($P<0.01$) by immersion at 42℃. The activity decreased from 0.92±0.08 to 0.44±0.08 U/gHb ($P<0.01$) and from 1.08±0.14 to 0.54±0.09 U/gHb ($P<0.05$), at 39℃ and 25℃, respectively (**Table 1**). At 42℃, erythrocyte sorbitol levels increased from 32.7±5.8 to 43.7±8.8 nmol/gHb ($P<0.05$,**Fig. 1**).

In vitro study

Epinephrine added in vitro in the presence of 8 mmol/l glucose produced dose-dependent increments of the erythrocyte AR activity and sorbitol content over the concentration range of 0 to 5.0μmol/ l (**Fig. 2,3**). Effect of incubation temperature on the erythrocyte AR activity is shown in **Fig. 4**. No changes were observed at 39℃. At 25℃, a transient decrease was detected after 5 min incubation, and then slightly increased above the initial level. At 42℃, the activity showed no changes 5 min later, however, obviously increased after 10 and 30 min incubation.

Table 1 Effect of immersion temperature on the activity of erythrocyte aldose reductase

Water temperature (°C)	Before (U/gHb)	After (U/gHb)
25	1.08±0.14	0.54±0.09*
39	0.92±0.08	0.44±0.08**
42	1.09±0.19	1.50±0.25**

Enzyme activities were measured before and after the water immersion for 10 min at 25°C, 39°C and 42°C. Data are expressed as mean± SEM.

*p<0.05, **p<0.01 v.s. Before.

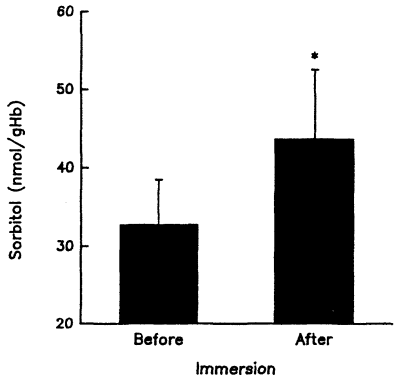

Fig. 1 Change of erythrocyte sorbitol levels after water immersion for 10 min at 42°C. Data are expressed as mean +SEM. * P <0.05 Before v.s. After

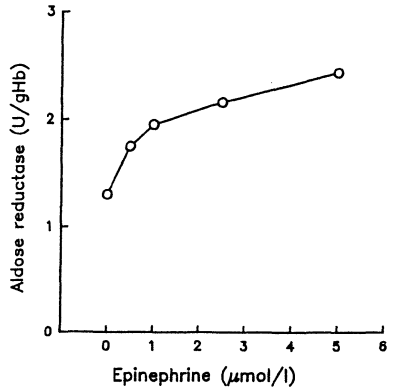

Fig. 2 Effect of epinephrine on erythrocyte aldose reductase activity.
Erythrocytes were incubated with different concentrations of epinephrine for 30 min at 39°C. Means of three experiments are shown.

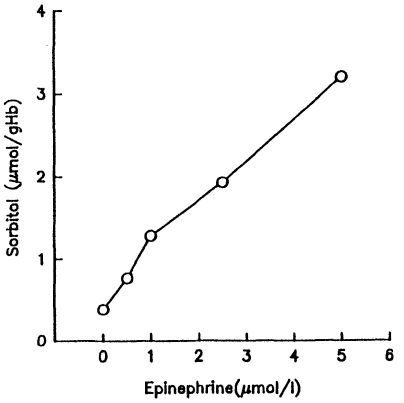

Fig. 3 Effect of epinephrine on erythrocyte sorbitol levels.
Erythrocytes were incubated with different concentrations of epinephrine for 30 min at 39°C. Means of three experiments are shown.

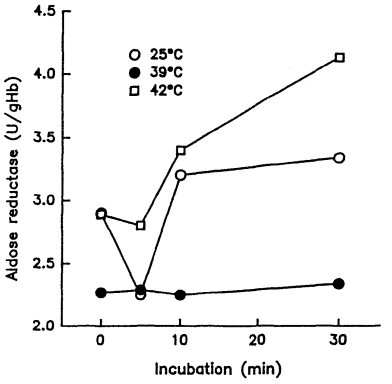

Fig. 4 Effect of incubation temperature on erythrocyte aldose reductase activity.
Erythrocytes were incubated at 25°C, 39°C and 42°C for 30 min.
Means of two experiments are shown.

DISCUSSION

The polyol pathway plays an important role in the development of complications in diabetes, and elevated AR activity in erythrocytes from diabetic patients has been reported[7),12)]. Activation of erythrocyte AR in man after oral glucose intake was reported by Lyons *et al.*[13)]. Glucose consumption led to a transient activation of this enzyme, which paralleled the rise and subsequent fall in blood glucose concentrations. In the present study (data not shown) and in a previous study[14)], no significant changes in the levels of blood glucose were observed after immersion at 25℃, 39℃ and 42℃ for 10 min ; therefore, it is unlikely that blood glucose level altered the AR activity during heat stress.

According to Hotta *et al.*[3)], epinephrine increased the sorbitol content of the rabbit retina, which was markedly reduced by AR inhibitor. In the present study, epinephrine increased the AR activity and sorbitol content in erythrocytes. From these findings it is thought that epinephrine can augment the AR activity in vitro. We observed the elevation of the plasma epinephrine level only after immersion at 42℃[14)]. Thus, one of the reasons for increased enzyme activity after immersion at 42℃ is suggested to be the raised epinephrine level.

An elevated level of extracellular sodium chloride was reported to increase the AR activity and the sorbitol level in renal medullary cells to maintain osmotic pressure[15)]. Plasma renin and ADH activities were increased after immersion at 42℃ but were suppressed at 25℃[14)]. This means that not only static water pressure but also water temperature is crucial for extracellular water distribution. A change in the extracellular sodium concentration, which is responsible for osmotic pressure, is thought to be another important factor to determine this enzyme activity.

In a preliminary study, under the same experimental conditions, we found that rectal temperature increased from 36.94±0.07℃ to 37.73±0.07℃ ($P<0.05$) only after the immersion at 42℃. Therefore, enhancement of this enzyme activity by heat stress is thought to occur not only on the peripheral skin surface but in the whole body. Since no significant hormonal changes were observed at 39℃[14)],. further experiments are required to elucidate the mechanism of this heat effect.

Although rectal temperature did not change very much under the present conditions, we examined direct effects of incubation temperature on the erythrocyte AR activity at 25℃, 39℃ and 42℃. Apparently, the enzyme activity increased at 42℃ and no change was observed at 39℃. These results also suggest the possibility that at higher temperature, this enzyme activity could be augmented. At 25℃, the enzyme activity fell after 5 min incubation by unknown mechanisms, however, it is assumed that lower incubation temperature might impair the enzyme activity transiently.

Water immersion is a useful technique for loading heat stress in humans and changes observed in the present study may also occur under other conditions such as in saunas or occupational environments. It is hypothesized that an increment of AR activity by heat stress may lead to development of diabetic complications and epinephrine possibly

plays an important role for this mechanism.

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