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# Glutathione Related Enzyme Activities in Spontaneous Hypertensive Rat Heart

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**It has been reported that oxygen radicals are involved in the development of tissue injury in hypertension. To prevent oxidative stress, there are antioxidant systems inside the cells such as superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reducatase (GR), catalase (CAT) and glutathione S-transferase (GST). In this study changes in these antioxidant activities were estimated in the outer wall of the left ventricles from spontaneously hypertensive rats (SHR), stroke prone SHR (SHRSP) and normal Wister Kyoto rats (WKY). The activities of manganese-superoxide dismutase (Mn-SOD), which localizes in mitochondria and GST were lower in the left ventricles of SHR and SHRSP compared to those in WKY. Slight decrease in the GPX activity was observed in the left ventricles from SHR and SHRSP. On the other hand, the activity of GR and catalase was not different in them. The effect of Nicardipine, a calcium channel blocker, on these antioxidant activities was also esimated. Treatment of these rats with nicardipine (150 mg/kg/day) for 4 weeks improved blood pressure, from 176±10 mmHg to 140±8 mmHg in SHR (n = 5), from 201±11 mmHg to 167±5 in SHRSP (n = 5), respectively, and restored the activities of Mn-SOD, GST and GPX. Collectively, these results suggest that oxidative stress in hypertensive rat heart causes supression of antioxidant activities, which may contribute to myocardical injury, and nicardipine plays a cardioprotective role to reduce the oxidative stress in hypertensive heart.**

Key words : antioxidants, calcium channels, hypertension.

## Introduction

It has been reported that oxygen radicals play a critical role in the pathogenesis of hypertension (1). Cardiac hypertrophy, a major underlying cause of heart disorders such as myocardial infarction and cardiac arrythmya (2) is formed when increased external stimuli such as hemodynamic overload and neurohumoral factors are continuoursly imposed on cardiac myocardium (3-6). These findings were affirmed by studies on hypertension using animal models having shown that hypertension linked to anatomical alterations and endothelial dysfunction in heart to lead to myocardial hypertrophy (3).

In hypertension, hemodynamic overload can induce myocardial hypertrophy. Cardiac hypertrophy is associated with myocardial ischemia due to disturbances in coronary physiology (1). Under this condition oxygen radicals are produced by xanthine oxidase (XO) in the endothelium (6-9). In addition to the hemodynamic overload, the roles of oxygen radicals have been emphasized in the progression of vascular injury in hypertension (10).

To scavenge oxidants, there are defense systems inside the cells. Superoxide dismutase (SOD) scavenges superoxide to form $H_2O_2$. There are two isozymes of SOD inside the cell. Cu, Zn-SOD presents in cytosol and Mn-SOD in mitochondria. These two isozymes are believed to detoxify superoxide in a similar manner. Especially, when mitochondrial respiratory chains leak a large amount of superoxide anion radicals which chain react with membrane phospholipid to develop lipid peroxidation; Mn-SOD can be induced and catalyzes superoxide detoxification within mitochondria. $H_2O_2$, which is formed by the catalytic reaction of SOD from $H_2O$ and superoxide, is far more toxic than superoxide and can permiate cell membranes. It is detoxified by glutathione peroxidase (GPX) or catalase. GPX uses glutathione as a substrate. Glutathione reductase (GR) converts oxidized glutathione by GPX to reduced glutathione and NADPH to NADP. GPX and GR are important in the redox cycle of NADP/NADPH. Glutathione S-transferase detoxifies xenobiotics to form glutathione S-conjugates.

Changes in the activities of antioxidants have been reported in SHR heart (11-13) or kidney (14). However, the data were not consistent nor contributable to understand the pathological significance of antioxidants in

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hypertension. Furthermore, the regulation of them in hypertensive tissues in response to oxidative stress is not clear. In the present study, the activity of SOD and GSH related enzymes was estimated in the outer wall of the left ventricles from spontaneously hypertensive rat (SHR), stroke prone SHR (SHRSP) and Wister Kyoto rat (WKY) hearts. In addition, the effect of nicardipine, a $Ca^{2+}$ antagonist, on the activity of these antioxidant enzymes was studied.

## Materials and Methods

*Materials*

Nicardipine was purchased from Yamanouchi Pharmaceutical Co., Tokyo, Japan. Glutathione reductase was from Pharmacia Fine Chemicals (Uppsala, Sweden).

*Animals and blood pressure measurements*

Six week old, male WKY, SHR, SHR of the Izumo colony (WKY/Izm, SHR/Izm, SHRSP/Izm) were obtained from the Disease Model Cooperative Research Association, Kyoto, Japan. A group of 2 to 3 rats was housed in a cage in an air-conditioned room at 24±1℃ , humidity of 65±5 %, with lights on 12 hours per day (7:00 a.m.-7:00 p.m.) at the Laboratory Animal Center for Biomedical Research Center, Nagasaki University School of Medicine. 10 of each group were divided into two group. Five rats of each group were fed with the diet containing nicardipine in a dose of 0.1% (wt/wt) (27), for 4 weeks (from 8 to 12 weeks of age). Another 5 rats of each group was given the diet with no drug served as controls. Systolic blood pressure was measures indirectly using an electrosphy gmomanometer (PS-2000, Riken Kaihatsu, Tokyo) after prewarming the whole body for 10 min at 38 ℃ to dilate the caudal tail artery.

One day after measuring systolic blood pressure in the 12 weeks-old animals, the rats were anesthetized with pentobarbital sodium (50 mg/kg body weight IP). The hearts were excised and washed with ice-cold isotonic solution (physiologic saline solution) and the outrer wall of the left ventricles was separated and divided in two pieces, one for protein and GSH assay, and another for gene expression.

*Enzyme Assay*

After homogenization of the excited left ventricles in 5 vol of ice-cold phophate buffered saline (0.9 vol of 0.154 NaCl and 9 vol of 0.1 M $NaH_2PO_4/Na_2HPO_4$, pH 7.4) in a Polytron homogenizer (KINEMATICA), the homogenate was centrifugated at 13,000 x g for 10 min. The supernatant was stored at −80℃ before use. The activity of SOD was estimated according to the method described by McCord and Fridovich (McCord and Fridovich. 1969). The activity of catalase, GST, GPX and GR was estimated photometrically according to the method described by Beutler (Beutler, 1986). 1-Chloro-2,4-dinitrobenzene and glutathione (GSH) were used as substrate for GST and changes in the absorbance at 412 nm was esimated. GSH and NADPH were used for the estimation of GPX activity, and the decreasing of NADPH was monitored at 340 nm. Glutathione disulfide and NADPH were used for the estimation of GR activity and decreasing of NADPH was monitered. Hydrogen peroxide was used for the estimation of catalase activity and change in the absorbance at 240 nm was estimated. One unit of enzyme activity was expressed as 1 $\mu$mol substrate changed/min. The total SOD activity was estimated by the cytochrome c reduction inhibition assay. The amounts of SOD required to inhibit the rate of reduction by 50% was defined as one unit of activity. The Mn-SOD which activity was obtained by substracting Cu, Zn-SOD activity is abolished by KCN from the total SOD activity.

*Statistical analysis*

The data are given as the mean±SD. Differences were calculated with Student's *t*-test.

## Results

*Changes in the blood pressure and heart weight*

Table 1 shows characteristics of the blood pressure, body weight and heart weight in hypertensive rats. The blood pressure was significantly more elevated in SHR (n = 5) than WKY (n = 5) (178±11.8 mmHg vs 115±3.5 mmHg, mean±SD, $p<0.05$). SHRSP rats were more severe (219 ±12.9 mmHg). Ratio of heart weight/body weight (mg/g) increased in SHR (5.25±0.01) and SHRSP (5.31±0.01) compared to that in WKY (4.04±0.03).

*Effect of nicardipine on the blood pressure and heart weight*

Treatment was administrated for 4 weeks and the effect of nicardipine on the blood pressure and heart weight was estimated. Nicardipine had no apparent effect on the blood pressure, body and heart weight in WKY rats. It improved the levels of blood pressure of SHR from 176±9.6 mmHg to 140±7.9 mmHg ($p<0.05$) and those of SHRSP from 201±10.8 mmHg to 167±4.5 mmHg ($p<0.05$). Nicardipine also decreased the herat weight/body weight ratio in SHR (5.12±0.02 vs 5.25±0.01, $p<0.05$), SHRSP (5.02±0.01 vs 5.31±0.01, $p<0.05$), respectively.

**Table 1.** Effect of nicardipine on body weight, blood pressure, and heart weight in hypertensive rats.

| Rat | Status | Body weight (g) | Systolic blood pressure (mmhg) | | Heart weight (mg) | Heart weight/Body weight (mg/g) |
|---|---|---|---|---|---|---|
| | | | before | after | | |
| WKY | control(5) | 240.0± 7.5 | 115± 3.5 | 114±6.5 | 970±3.3 | 4.04±0.03 |
| | nicardipine(5) | 234.4± 5.4 | 123±10.9 | 116±8.2 | 946±2.4 | 4.03±0.01 |
| SHR | control(5) | 177.8± 5.7 | 178±11.8* | 169±5.5 | 934±3.2* | 5.25±0.01 |
| | nicardipine(5) | 175.6± 4.4 | 176± 9.6 | 140±7.9** | 900±2.2** | 5.12±0.02** |
| SHR-SP | control(5) | 178.8±11.8 | 219±12.9* | 223±8.4 | 950±6.4* | 5.31±0.01 |
| | nicardipine(5) | 179.4±10.3 | 201±10.8 | 167±4.5** | 900±5.1** | 5.02±0.01** |

Rats were treated with nicardipine (150mg/kg/day) for four weeks.
The numbers in parentheses indicate the numbers of animals in each group.
*, $P<0.05$ vs WKY
**, $P<0.05$ vs control of each group. Values are expressed as the mean ± SD.

*Antioxidant activity*

Change in the activity of antioxidants was estimated in the left ventricles. Decrease in the activity of Mn-SOD was observed in SHR compared to that in WKY (60.5 ± 1.9 mU/mg protein vs 71.1 ± 3.2, $p<0.05$). The decrease in Mn-SOD activity was more in SHRSP (51.9 ± 2.4 mU/mg protein) (Fig. 1). The activity of GST also decreased in SHR (26.9 ± 0.9 mU/mg protein) and more in SHRSP (22.3±3.1 mU/mg protein) compared to that in WKY (3 1.9±1.0 mU/mg protein) (Fig. 2). The activity of GPX was slighty reduced in SHR and SHRSP, although being not significant (Fig. 3). The activity of other antioxidants such as Cu, Zn-SOD (Fig. 4), catalase (Fig. 5) and GR (Fig. 6) was not different in these groups.

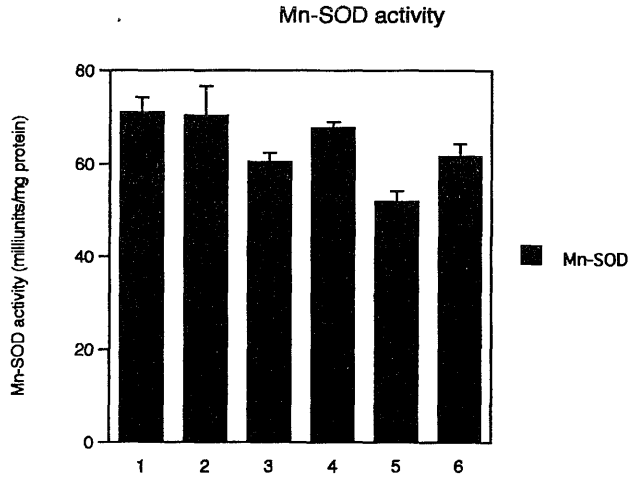


**Figure 1.** The activity of Mn-SOD in the left ventricle of rat hearts. The activity of Mn-SOD was estimated in the outer wall of left ventricles from rat hearts with or without the treatment with nicardipine for four weeks. The homogenized tissue was centrifugated and the supernatant was used as material for the estiamation of enzyme activities. The Mn-SOD activity was measured as described in Materials and Methods. Values are mean±SD of five samples. Lanes 1 and 2, WKY ; lanes 3 and 4, SHR ; lanes 5 and 6, SHRSP. Lanes 2, 4 and 6, treatment with nicardipine (150 mg/kg/day).

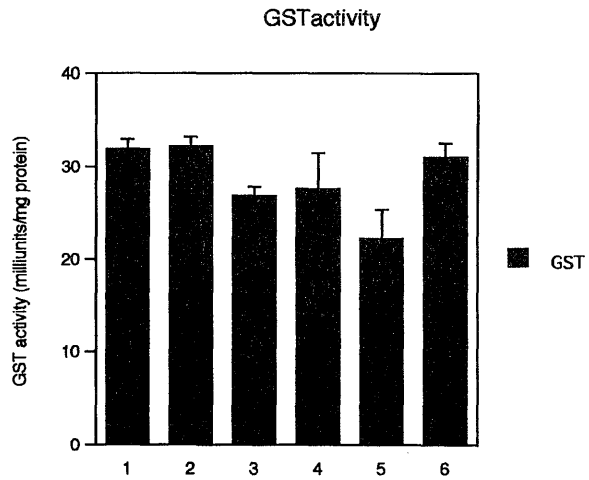


**Figure 2.** The activity of GST. The activity of GST was estimated in the outer wall of left ventricles from rat hearts with or without the treatment with nicardipine for four weeks. The GST activity was measured as described in Materials and Methods. Values are mean±SD of five samples. Lanes 1 and 2, WKY ; lanes 3 and 4, SHR ; lanes 5 and 6, SHRSP. Lanes 2, 4 and 6, treatment with nicardipine (150 mg/kg/day).

*Effect of nicardipine on the antioxidant activities*

Nicardipine had no apparent effect on the antioxidant activities in WKY. It increased the reduced activity of Mn-SOD in SHR left ventricles by 112% and 119% in SHRSP (Fig. 1). Similar effect of nicardipine was observed for GST activity in SHRSP left ventricles (increase by 139%) (Fig. 2). Restoration of the GPX activity by nicardipine was 108% in SHR and SHRSP (Fig. 3).

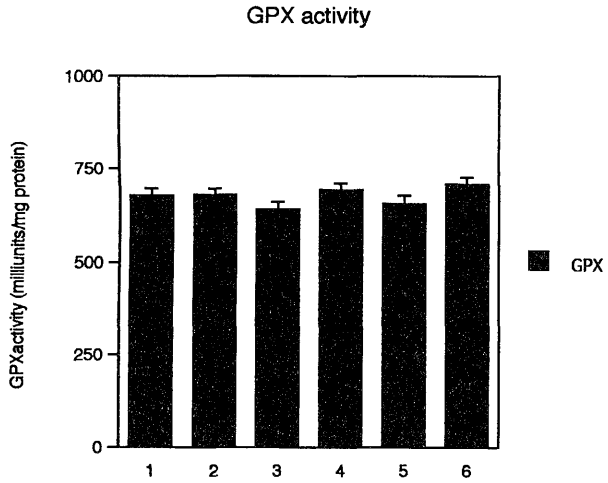


**Figure 3.** The activity of GPX. The activity of GPX was estimated in the outer wall of left ventricles from rat hearts with or without the treatment with nicardipine for four weeks. The GPX activity was measured as described in Materials and Methods. Values are mean±SD of five samples. Lanes 1 and 2, WKY ; lanes 3 and 4, SHR ; lanes 5 and 6, SHRSP. Lanes 2, 4 and 6, treatment with nicardipine (150 mg/kg/day).

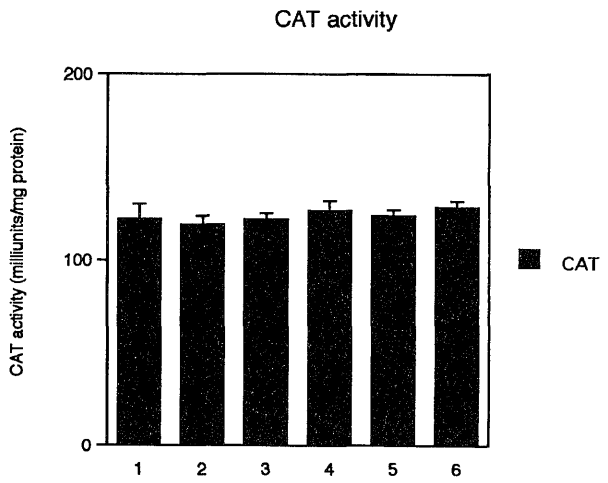


**Figure 5.** The activity of catalase. The activity of catalase was estimated in the outer wall of left ventricles from rat hearts with or without the treatment with nicardipine for four weeks. The catalase activity was measured as described in Materials and Methods. Values are mean±SD of five samples. Lanes 1 and 2, WKY ; lanes 3 and 4, SHR ; lanes 5 and 6, SHRSP. Lanes 2, 4 and 6, treatment with nicardipine (150 mg/kg/day).

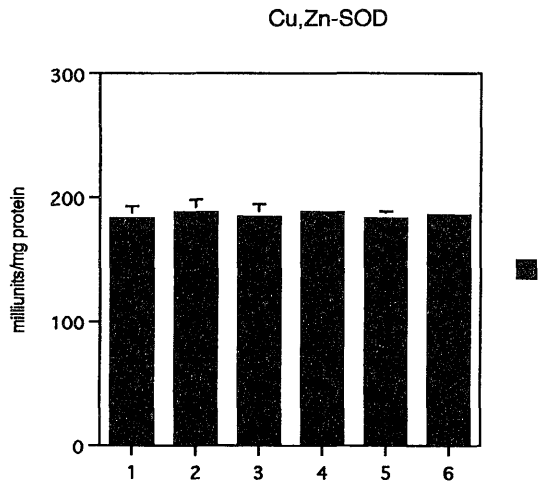


**Figure 4.** The activity of Cu,Zn-SOD. The activity of Cu,Zn-SOD was estimated in the outer wall of left ventricles from rat hearts with or without the treatment with nicardipine for four weeks. The Cu,Zn-SOD activity was measured as described in Materials and Methods. Values are mean±SD of five samples. Lanes 1 and 2, WKY ; lanes 3 and 4, SHR; lanes 5 and 6, SHRSP. Lanes 2, 4 and 6, treatment with nicardipine (150 mg/kg/day).

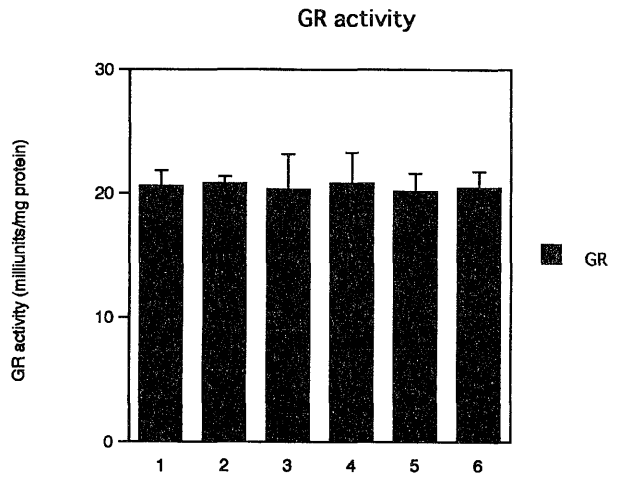


**Figure 6.** The activity of GR.The activity of GR was estimated in the outer wall of left ventricles from rat hearts with or without the treatment with nicardipine for four weeks. The GR activity was measured as described in Materials and Methods. Values are mean±SD of five samples. Lanes 1 and 2, WKY ; lanes 3 and 4, SHR ; lanes 5 and 6, SHRSP. Lanes 2, 4 and 6, treatment with nicardipine (150 mg/kg/day).

## Discussion

Production of reactine oxygen species in SHR has been reported in microvascular walls (15, 16). Cardiomyocite hypertrophy in hypertension can be induced by oxidative stress, hemodynamic overload, and neurohumoral factors. These external stimuli are generally transduced into the cells through intracellular signal transduction cascade (17).

Supression of Mn-SOD activity together with an increase in mitochondrial lipid peroxidation was first reported by Ohtsuki in SHR kidney (18). This suggests that mitochondrial damage and superoxide leak are present in hypertensive tissues. In the present study, the activity of Mn-SOD but not Cu, Zn-SOD decreased in SHR hearts and more in SHRSP hearts. The data are in good agreement with the previous report (18) and suggest that the impairment of mitochondrial function in hypertensive hearts. Mn-SOD is responsive to oxidative stress. The expression of Mn-SOD is stimulated in respons to oxidative stress and cytokines (19). Whereas, the expression of Cu, Zn-SOD is not responsive to any stimulation, except for glycation of Cu, Zn-SOD protein in diabetic conditions (20). The supression of Mn-SOD activity observed in hypertensive hearts may be caused by oxidative stress in hypertension. A possible explanation for an increase in oxidative stress is a high activity of xanthine oxidase in hypertension. Miyamoto has reported that vasorelaxation can be induced by the treatemnt with xanthine oxidase inhibitors (9), which improve endothelial vasodilator function mediated by nitric oxide (8).

Intracellular GPX is a selenium-containing enzyme and its activity is stable under physiological and pathological conditions (21, 22). The regulation of GPX is not clear, however, decrease in the GPX activity in hypertensive hearts may reflect the oxidative stress in hypertension.
Cardioprotective effect of nicardipine inhibition of the inward transport of calcium by nicardipine induces cardioprotective effect. It prevents mitochondrial calcium overload and concequently preserve the structure and function of myocardial cells while protecting against the deleterious effects of hypoxia and ischemia (23). Nicardipine calcium bockers also restore the impaired basoreceptor reflex control (24). In addition, the effect of nicardipine on coronary hemodynamics and myocardial oxygen consumption contributes to a cardioprotective effect. Decrease in the lipid peroxidation by nicardipine has been reported, suggesting the antioxidant activity of calcium blockers (25, 26).

In the present study, the activity of Mn-SOD, GPX and GST was normalized by the treatment with nicardipine. Although the regulation of the activity of these enzymes was not studied, the expression of genes encoding these enzymes may be regulated by similar transcription factors, which are stimulated when myocardial cells are exposed to oxidative stress and can function to downregulate the expression of these antioxidant enzymes.

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