Hepatic changes in adenine nucleotide levels and adenosine 3'-monophosphate forming enzyme in streptozotocin-induced diabetic mice

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(Received December 15, 2007; Accepted January 7, 2008)

ABSTRACT — To elucidate the pathophysiological significance of adenosine 3'-monophosphate (3'-AMP) forming enzyme in mice, the effect of streptozotocin (STZ) on the enzyme activities and adenine nucleotide levels in the ICR mice (4-week-old) liver was examined. After 2 weeks, treatment with a single dosage of STZ (100, 150 or 200 mg/kg i.p.) induced a dose-dependent hyperglycemia and hypoinsulinemia but had no effect on serum alanine aminotransferase activity, indicating that STZ generated type 1 diabetes without hepatitis. In the diabetic liver, the activities of superoxide dismutase (SOD), catalase and ATP levels decreased, and the microsomal CYP2E1 activity increased. Changes of these biological activities might disrupt the cellular homeostatic balance of reactive oxygen species (ROS) production. The activities of 3'-AMP forming enzyme, one of the ribonucleases, in hepatic homogenates were not altered. However, in the STZ 200 mg/kg group, the cytosolic forming enzyme activities were enhanced, and inversely, the mitochondrial activity was reduced significantly, indicating that the decrease in the mitochondrial activity may be accelerated by development of diabetes due to the decrease in the antioxidant defense system and/or increase in ROS production. With the decrease in the 3'-AMP forming enzyme activity, the levels of 3'-AMP, a P-site inhibitor of adenylate cyclase, in mitochondrial were significantly reduced. These results obtained suggested that change in the mitochondrial 3'-AMP forming enzyme activity might reflect the pathophysiological change of mitochondrial function with the development of diabetes. Our results also suggested that change in cytosolic enzyme activity might serve as a new biomarker of oxidative stress because significant negative correlation between the activities of cytosolic 3'-AMP forming enzyme and SOD was found in the early stage of diabetes.

Key words: Adenosine 3'-monophosphate, Diabetes, Mouse, Liver, Streptozotocin

INTRODUCTION

Intracellular adenosine 3'-monophosphate (3'-AMP) is pharmacologically recognized as an intracellular P-site inhibitor of adenylate cyclase (Bushfield et al., 1990; Iwatsubo et al., 2006). The 3'-AMP forming enzyme, one of the ribonucleases (RNases), is known to exist not only in cytosol of some organs in rats (Bushfield et al., 1990; Fujimori et al., 2004) and mice (Fujimori et al., 2001) but also in mitochondria of rat liver (Fujimori et al., 1998; Fujimori and Pan-Hou, 2002). However, the pathophysiological roles of 3'-AMP and its forming enzyme have not yet been elucidated.

Streptozotocin (STZ) is known to exert toxic effects not only on pancreatic islet β-cells but also on other organs including liver (Weiss, 1982; Kume et al., 1994; Haluzík and Nedvidková, 2000; Kume et al., 2004). The liver is a major target of insulin action, and plays an important role in maintaining blood glucose levels (Dhabhi et al., 2003; Parker et al., 2004). Bushfield et al. (1990) demonstrated that STZ-induced increase in 3'-AMP levels in rat liver could be largely reversed by insulin treatment. From this finding, STZ may serve as a tool for elucidating the pathophysiological roles of 3'-AMP forming enzyme in liver. Our interest is to know whether diabetes induced by STZ would influence the 3'-AMP forming enzyme activities in the cytosol and mitochondria from mouse liver.

In this study, we examined the effect of STZ on adenine nucleotide levels and the 3'-AMP forming enzyme activity in mouse liver. Here we report that decrease in
mitochondrial 3'-AMP forming enzyme activity in STZ-induced diabetic mouse liver was associated with the increase in cytosolic 3'-AMP forming enzyme activity, and speculated that change in the mitochondrial 3'-AMP forming enzyme activity may reflect the pathophysiological change of mitochondrial functions due to reactive oxygen species (ROS) production.

**MATERIALS AND METHODS**

**Materials**

3'-AMP, adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (5'-AMP), adenosine (Ado) and polyadenylic acid [poly(A)] were purchased from Yamasa Shoyu (Chiba, Japan). STZ and chloroacetaldehyde were obtained from Sigma-Aldrich (St. Louis, USA). The Transaminase CII-test Wako, SOD test Wako, Glucose CII-test Wako and p-nitrophenol were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 

**Animals**

All laboratory animals for these experiments were treated according to the Guidelines of the Committee for Ethical Use of Experimental Animals at Setsunan University, Japan. Mice were housed in polycarbonate cages in an animal room air-conditioned at a temperature of 25 ± 2°C and a relative humidity of 55 ± 10% with 15 times ventilation per hr under a 12-hr light and dark cycle, and allowed free access to regular chow diet and tap water throughout the experimental period for 2 weeks. Mice were randomly assigned to treatment groups (5 animals/group) and acclimated for 7 days.

**Treatment of mice with STZ**

Mouse blood glucose level was measured before STZ injection. After fasting for 24 hr, STZ (100, 150 or 200 mg/kg body weight), freshly dissolved in 0.05 M sodium citrate buffer (pH4.5), was injected intraperitoneally at around 13:00. Mice in the control group were given vehicle alone. At 2 weeks after STZ-injection, the blood was withdrawn from the abdominal aorta of the mouse under ether anesthesia between 13:00 and 16:00, and the serum was obtained by centrifugation at 1,500 x g for 10 min.

**Blood and serum biochemistry**

Blood glucose level was measured using Glucose Pilot strips (Aventir Biotech, LLC., CA, USA) covered with a drop of blood obtained by a tail-ven puncture just before ether anesthesia. Serum insulin level and the activity of serum alanine aminotransferase (ALT) were measured using an immunoassay kit (Morinaga Institute of Biological Science, Yokohama, Japan) and a transaminase CII-test Wako, respectively. Serum levels of ketone bodies (β-hydroxybutyril acid and acetocetic acid) were measured using a Ketolex kit (Sanwa Kagaku Co., Ltd., Aichi, Japan), based on the method described by Williamson et al. with slight modifications (Uno et al., 1987). The levels of ketone bodies were calculated by the sum of the amounts of β-hydroxybutyril acid and acetocetic acid.

**Preparation of crude mitochondria and microsome from mouse liver**

All procedures were performed at 0-4°C. The liver was quickly removed from the mouse and weighed. The liver (1.0 g) was homogenized immediately after removal in 9.0 ml of 20 mM Tris-HCl buffer (pH 7.5) containing 0.25 M sucrose, 1 mM EDTA and 5 mM 2-mercaptoethanol. Part of the homogenate obtained was kept at -40°C until use and used as a homogenate enzyme solution. The residual homogenate was then centrifuged at 600 x g for 15 min. The supernatant was centrifuged twice at 9,000 x g for 15 min to separate mitochondria. The pellet was suspended in the same Tris-HCl buffer described above. The suspension obtained was kept at -40°C until use, and used as a crude mitochondrial enzyme solution. The residual supernatant obtained was further centrifuged twice at 210,000 x g for 40 min. The pellet was suspended in the same Tris-HCl buffer, kept at -80°C, and used as a microsomal enzyme solution. The supernatant obtained was also kept at -40°C and used as a cytosolic enzyme solution. Protein was determined by the method of Bradford using bovine serum albumin as a standard (Bradford, 1976).

**Determination of liver glycogen content**

Liver glycogen content was measured according to the method described by Suzuki et al. with a slight modification (Suzuki et al., 2001). Glycogen isolated from frozen liver was hydrolyzed to glucose with amyloglucosidase and measured as glucose equivalent using Glucose CII-test Wako. Glycogen content is expressed as μmol of
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Determination of superoxide dismutase (SOD) and catalase activities

SOD activity was measured using a SOD test-Wako according to the method based on the inhibition by SOD of nitroblue tetrazolium reduction due to superoxide (Yamanaka et al., 1979). The SOD activity is expressed as the inhibition (%).

Catalase activity was measured using the method described by Aebi with modifications (Aebi, 1974). The reaction mixture consisted of 50 µl of 1 M Tris-HCl (pH 8.0) containing 5 mM EDTA, 30 µl of distilled water and 900 µl of 10 mM H₂O₂, was preincubated at 37°C. After 10 min incubation, 20 µl of liver samples were added to the reaction mixture, and the absorbance was monitored for 1 min at 240 nm. The change in absorbance is proportional to the breakdown of H₂O₂. The catalase activity is expressed as units/mg protein.

Determination of CYP2E1 activity

CYP2E1 activity was assayed by p-nitrophenol (PNP) hydroxylating activity in terms of 4-nitrocatechol formation according to the method described by Koop et al. with a slight modification (Koop et al., 1997). The reaction mixture (1,000 µl) consisted of 0.1 M potassium phosphate buffer (pH 6.8) containing 1 mM NADPH, 0.1 mM PNP and 40 µl of various microsomal fraction. The reaction was terminated by adding 200 µl of 1.5 M perchloric acid. After 100 µl of 0.1 M NaOH was added to 1,000 µl of the supernatant at 9,000 x g for 4 min, the absorbance at 546 nm was measured with a spectrophotometer. The CYP2E1 activity is expressed as nmol/min/mg protein.

Determination of Adenine Compounds by HPLC

Extraction of adenine compounds in the liver with perchloric acid was carried out according to the method previously described (Fujimori and Pan-Hou, 1998). The acid-soluble compounds were treated with 50% chloroacetaldehyde for fluorescence detection of 1,N-etheno-derivatives of ATP, ADP, 5'-AMP, 3'-AMP, and Ado. The derivatized Ado, 3'-AMP and 5'-AMP were determined by a reversed-phase HPLC using a column of Chromatorex ODS (Fujimori et al., 1998), and the derivatized ATP, ADP and 5'-AMP were determined using a column (125 x 4.6 mm i.d.) of Hitachi gel No. 3013-N as an anion exchanger (Fujimori et al., 1991). The eluate was detected with an HPLC system consisting of an Intelligent HPLC pump (Jasco 880-FP, Japan), an Intelligent spectrofluorometer (Jasco 820-FP, Japan) and an integrator (Jasco 829-IT, Japan). The adenine compound levels are expressed as μmol/g wet weight.

Determination of 3'-AMP forming enzyme activity

3'-AMP forming enzyme activity was determined according to the procedure described previously (Fujimori et al., 1998). The reaction mixture (55 µl) consisted of 0.1 M acetate buffer (pH 5.6) containing 20 mM EDTA, 25 µg poly (A) and 5 µl of various crude enzyme solution. After incubation of the mixture at 37°C for 60 min, 55 µl of 15 mM ZnCl₂ in 0.5 M perchloric acid were added to stop the reaction. The enzymatic products were derivatized with chloroacetaldehyde, and the derivatized compounds were analyzed using a column of Chromatorex ODS maintained at 45°C. The 3'-AMP forming enzyme activity is estimated by the sum of the activity to produce 3'-AMP and Ado, and is expressed as nmol/60 min/mg protein.

Statistical Analysis

All experimental results are expressed as mean ± S.E. Statistical analysis was carried out by Student’s t-test or Dunnett’s test (Dunnett, 1964). p values < 0.05 were considered to be significant. Analysis was performed using Excel software.

RESULTS

The body weights of the STZ 200 mg/kg group were significantly suppressed to ca. 86% of those of the control group as shown in Table 1. In the STZ 200 mg/kg group, the relative liver weight was significantly higher, and the relative epididymal fat pad weight was lower comparing with those in the control group (Table 1). Blood glucose levels were significantly enhanced in a dose-dependent manner (Fig. 1-A), and serum insulin levels were significantly decreased dose-dependently (Fig. 1-B). Serum ketone body levels of the STZ 200 mg/kg group were increased but no significance was found (Fig. 1-C).

The hepatic SOD activities in STZ 150 and 200 mg/kg groups were significantly suppressed to ca. 80% and 65% of those in the control group, respectively (Fig. 2-A). The catalase activity in the STZ 200 mg/kg group was significantly reduced to ca. 27% of control activity (Fig. 2-B). On the other hand, the hepatic CYP2E1 activity in the STZ 200 mg/kg group was significantly enhanced to ca. 1.3-fold of those in the control group (Fig. 2-C). However, no significant change in serum ALT activity was observed as shown in Fig. 2-D.

Under these experimental conditions mentioned above, the effect of STZ on the levels of adenine compounds

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Table 1. Effect of STZ on body weight and relative organ weight

<table>
<thead>
<tr>
<th>STZ (mg/kg)</th>
<th>Body weight (g)</th>
<th>Relative organ weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>0</td>
<td>32.8 ± 1.4</td>
<td>5.68 ± 0.30</td>
</tr>
<tr>
<td>100</td>
<td>33.9 ± 0.6</td>
<td>6.37 ± 0.12*</td>
</tr>
<tr>
<td>150</td>
<td>32.4 ± 0.8</td>
<td>6.97 ± 0.26**</td>
</tr>
<tr>
<td>200</td>
<td>28.2 ± 1.5*</td>
<td>7.11 ± 0.20**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E. a: 100 x organ weight (g)/body weight (g).
*p < 0.05 vs. control. **p < 0.01 vs. control.

Table 2. Effect of STZ on adenine compounds and glycogen levels in mouse liver

<table>
<thead>
<tr>
<th>STZ (mg/kg)</th>
<th>nmol/mg wet weight</th>
<th>Glycogen (μmol/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3'-AMP</td>
<td>Ado</td>
</tr>
<tr>
<td>0</td>
<td>0.033 ± 0.003</td>
<td>0.036 ± 0.006</td>
</tr>
<tr>
<td>100</td>
<td>0.030 ± 0.002</td>
<td>0.034 ± 0.004</td>
</tr>
<tr>
<td>150</td>
<td>0.033 ± 0.003</td>
<td>0.041 ± 0.003</td>
</tr>
<tr>
<td>200</td>
<td>0.024 ± 0.003</td>
<td>0.044 ± 0.005</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E. of five mice. *p < 0.05 vs. control. **p < 0.01 vs. control.
a: Total = [ATP+ADP+AMP].

and glycogen in mouse liver was examined. Hepatic glycogen content of the STZ 200 mg/kg group decreased to ca. 56% of the control value (Table 2). As shown in Table 2, hepatic ATP levels in the STZ 200 mg/kg group was significantly reduced to ca. 56% of control levels. The 5'-AMP levels of STZ 150 and 200 mg/kg groups were significantly increased up to ca. 1.7- and 1.8-fold, respectively. No significant changes in Ado, ADP and total adenine compound levels were observed between the control and STZ groups (Table 2). The ratio of 5'-AMP/ATP of the STZ 200 mg/kg group was ca. 3.3-fold higher than that of control. Hepatic 3'-AMP levels of the STZ 200 mg/kg group decreased to ca. 74% of the control levels (Table 2). On the other hand, hepatic mitochondrial 3'-AMP levels of the STZ 150 and 200 mg/kg groups were significantly suppressed to ca. 68 and 74% of the control levels, respectively (Table 3).

To study the mechanism of mitochondrial 3'-AMP level reduction, the effect of STZ on 3'-AMP forming enzyme activity in mouse liver was examined. No significant changes in the 3'-AMP forming enzyme activity in the crude homogenate were observed as shown in Fig. 3-A. The 3'-AMP forming enzyme activity in the crude mitochondria of the STZ 200 mg/kg group was significantly suppressed to ca. 60% of control values (Fig. 3-B).

Table 3. Effect of STZ on 3'-AMP levels in mouse hepatic mitochondria

<table>
<thead>
<tr>
<th>STZ (mg/kg)</th>
<th>3'-AMP level (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.17 ± 0.07</td>
</tr>
<tr>
<td>100</td>
<td>1.23 ± 0.11</td>
</tr>
<tr>
<td>150</td>
<td>0.79 ± 0.05**</td>
</tr>
<tr>
<td>200</td>
<td>0.87 ± 0.07*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E. of five mice. *p < 0.05 vs. control. **p < 0.01 vs. control.
Effect of STZ on mouse hepatic 3'-AMP forming enzyme

On the other hand, the cytosolic activity of the STZ 200 mg/kg group was significantly enhanced to ca. 1.8-fold compared with the control value (Fig. 3-C). In the control group, the mitochondrial 3'-AMP forming enzyme activity was ca. 81-fold higher compared with the cytosolic activity. However, in the STZ 200 mg/kg group, the mitochondrial activity was decreased to ca. 26-fold of the cytosolic one.

Next, the 3'-AMP and Ado degrading activities in mouse liver treated with 200 mg/kg STZ were evaluated. As shown in Table 4, the 3'-AMP degrading activity in mitochondrial fraction was ca. 35 higher than that in cytosolic fraction. No significant changes in cytosolic and mitochondrial 3'-AMP degrading activities were observed when a mouse was treated with 200 mg/kg STZ (Table 4). The Ado degrading activity was only found in cytosolic fraction, in which the activity was not affected by the STZ treatment (Table 4).

The effect of pCMBS on the 3'-AMP forming enzyme activities in cytosolic and mitochondrial fractions was examined. As shown in Fig. 4, the treatments with pCMBS without largely influenced the 3'-AMP forming activities in the cytosolic and mitochondrial fractions.

DISCUSSION

STZ is often used to generate animal models of type 1 diabetes through its toxic effects on pancreatic islet β-cells (Weiss, 1982; Kume et al., 1994; Kume et al., 2004). Under our experimental conditions, a dose-dependent typical hyperglycemia (Fig. 1-A) and hypoinsulinemia (Fig. 1-B) were observed, indicating that early stages of type 1 diabetes in mice were induced by a single administration of STZ after 2 weeks. During mice housing, proteinuria and glucosuria were detected (data not shown), suggesting that representative nephrotoxicity was elicited by STZ treatment. However, no change in serum ALT activity as shown in Fig. 2-D strongly indicated that 2 week-STZ treatment did not cause hepatitis with damage to hepatocellular membrane although STZ is known to exert acute hepatic changes including lipid peroxidation, mitochondrial swelling, and inhibition of hepatocyte proliferation before the elevation of the serum glucose levels (Kume et al., 2004).

STZ-induced hepatic toxicity is thought to be mediated by ROS which produces lipid peroxide in hepatocellular membrane (Ahn et al., 2006; Kayali et al., 2004; Raza et al., 2004). Cellular homeostatic balance of ROS including superoxide radical might be influenced by 1) CYP2E1 activity which catalyzes the formation of ROS during metabolizing endogenous compounds

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**Fig. 1.** Changes in blood glucose (A), serum insulin (B) and serum ketone body (C) levels in mice after a single i.p. injection of STZ. These data were obtained at 2 weeks after 100, 150 or 200 STZ mg/kg administration. Ketone body was calculated by the sum of the level of β-hydroxybutyric acid and acetoacetic acid. Value represents mean ± S.E. of five mice. **p < 0.01 vs. control.**

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such as fatty acids, lipid hydroperoxide, and ketone bodies into aldehydes (Tanaka et al., 2000; Mari and Cederbaum., 2001), 2) ATP-dephosphorylating activity which results in the formation of superoxide radical via supphcation of a substrate for xanthine oxidase (Nukatsuka et al., 1988; Nukatsuka et al., 1990), 3) mitochondrial respiration-linked ROS production (Raza et al., 2004), 4) antioxidant defending activity such as SOD and catalase activities (Low et al., 1997). The increases in CYP 2E1 activity (Fig. 2-C) and serum ketone body levels (Fig. 1-C) and decreases in hepatic ATP levels (Table 2) and the activities of SOD and catalase (Figs. 2-A and B) were observed under our experimental conditions. These biochemical changes may involve an increase of ROS production in the liver after STZ treatment. The increase in the formation of ROS induced by STZ might elicit mitochondrial dysfunction in the mice. Hypertrophy of hepatocytes, which were characterized by a prominent increase in a number of mitochondria (Hamperl, 1962) and a marked decrease in glycogen granules showing normal structure, is known to be induced in diabetic mice 4 to 12 weeks after a single administration of STZ (Kume et al., 1994). Hepatic hypertrophy (i.e., increase in relative liver weight) was observed in our STZ 200 mg/kg group (Table 1). However, 2-week-STZ treatments did not induce acute hepatitis because no change in serum ALT activity was observed (Fig. 2-D). Judging from the large decrease in hepatic ATP levels which are known to be predominantly produced via oxidation phosphorylation in mitochondria, there is no doubt that mitochondrial function was certainly suppressed by STZ (Table 2).

In general, RNase, one of the SH-enzymes, is known to exist as an active form and an inactive form complexed with RNase inhibitor in cells and tissues (Kumagai et al.,

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**Fig. 2.** Effect of STZ on activities of SOD (A), Catalase (B), CYP2E1 (C) and ALT (D)

Value represents mean ± S.E. of five mice. *p < 0.05 vs. control. **p < 0.01 vs. control.
Effect of STZ on mouse hepatic 3'-AMP forming enzyme

![Graph A](image)

![Graph B](image)

![Graph C](image)

**Fig. 3.** Effect of STZ on activities of 3'-AMP forming enzyme (s) in the crude homogenate fraction (A), mitochondrial fraction (B) and cytosolic fraction (C) of mouse liver. Data are expressed as mean ± S.E. of five mice. *p < 0.05 vs. control.

1991; Deutscher, 1993). The inactivation of RNase inhibitor, rich in SH residues, by SH reagents such as pCMBS causes the dissociation of the RNase-RNase inhibitor complex into active RNase and inactive inhibitor, in which SH residues are critical for the inhibition of RNase (Ferreras et al., 1995). RNases do not hydrolyze RNAs to produce Ado directly. Conversion of 3'-AMP to Ado is catalyzed by acid phosphatase (Fujimori et al., 1998), and the yielded Ado is converted to inosine, which is not determined by our HPLC, by Ado deaminase. Therefore, the net 3'-AMP forming enzyme activity is estimated by the sum of the activity to produce 3'-AMP and Ado. Treatment with 200 mg/kg STZ did not significantly affect 3'-AMP forming activity in the liver homogenates but the activity in mitochondrial fraction was largely inhibited (Fig. 3-B). The forming activity in cytosol was increased along with

**Fig. 4.** Effect of pCMBS on 3'-AMP forming activities in the mitochondrial fraction (A) and cytosolic fraction (B) of mouse liver treated without (c) and with (w) pCMBS. To the reaction mixture for the determination of 3'-AMP forming enzyme activity described in METHODS were added 4 μl of 5.5 mM pCMBS. Data are expressed as mean ± S.E. of five mice.
Table 4. Effect of STZ on 3'-AMP and Ado degrading activities

<table>
<thead>
<tr>
<th>STZ (mg/kg)</th>
<th>Fraction</th>
<th>3'-AMP degraded (nmol/60 min/mg protein)</th>
<th>Ado degraded (nmol/60 min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Cytosol</td>
<td>83.8 ± 15.9 *</td>
<td>920 ± 106</td>
</tr>
<tr>
<td></td>
<td>Mitochondria</td>
<td>2925 ± 635</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>200</td>
<td>Cytosol</td>
<td>80.5 ± 6.2 *</td>
<td>1207 ± 92</td>
</tr>
<tr>
<td></td>
<td>Mitochondria</td>
<td>2724 ± 698</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Hepatic cytosol and mitochondria fractions were obtained at 2 weeks after 0 or 200 mg/kg STZ administration. 3'-AMP or Ado degrading activity was determined using 3'-AMP or Ado as substrate in place of poly(A) according to the procedure of determination of 3'-AMP forming enzyme activity described in METHODS. Values represent mean ± S.E. of five mice. *p < 0.01

The decrease of the forming activity in mitochondrial fraction (Fig. 3). The increase in cytosolic 3'-AMP forming enzyme activity (Fig. 3-C) may not arise from the activation of the enzyme via inactivation of RNase inhibitor under diabetes conditions because there was no significant difference in the forming activity both in cytosolic and mitochondrial fractions between the treatment without and with pCMBS (Fig. 4). In addition, no Ado degrading activity was detected in mitochondrial fraction (Table 4), and no significant difference in the 3'-AMP degrading activities both in cytosol and mitochondrial fractions was found between with and without STZ treatment. These results clearly suggested that the enhancement of the 3'-AMP forming enzyme activity in the cytosol (Fig. 3-C) may be at least partly due to the leakage of the enzyme activity from mitochondria probably via ROS-mediated membrane damage.

In this study, we demonstrated that mitochondrial 3'-AMP levels and its forming enzyme activity were significantly influenced by development of diabetes induced by STZ (Table 3 and Fig. 3-B). Therefore, much attention should be attracted to elucidate the pathophysiological roles of the mitochondrial 3'-AMP forming enzyme system. A significant negative correlation between cytosolic 3'-AMP forming enzyme and SOD (r = -0.52, p < 0.05), calculated from the data shown in Figs. 3-C and 2-A, was observed. Therefore, cytosolic 3'-AMP forming enzyme activity in the liver might serve as a biomarker to estimate varying degrees of oxidative stress in the early stage of mouse diabetes.

ACKNOWLEDGMENTS

The authors are grateful to Miss Y. Nishioka, Miss Y. Onishi, Miss N. Sowa and Mr. H. Izumi of Setsunan University for their technical assistance.

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