Plasma Atrial Natriuretic Peptide Levels in Rabbits with Alloxan Monohydrate- induced Diabetes Mellitus

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SUMMARY

The effect of alloxan monohydrate-induced diabetes on the resting plasma atrial natriuretic peptide (ANP) level was investigated in 22 male New Zealand white rabbits. Alloxan monohydrate (100 mg/kg) dissolved in saline at a concentration of 50 mg/ml was administered by a single intravenous injection 3 months before the experimental analysis. The diabetic state was examined 72 h later by quantitative determination of blood glucose levels of >350 mg/dl. Beginning on day 3, 14 animals (Group 1) received a daily subcutaneous injection of 1 U insulin having moderate hyperglycemia (blood glucose concentration [BGC] between 300 and 400 mg/dl). Eight animals (Group 2; normoglycemic controls) received 3.2 U of insulin daily to maintain the BGC below 100 mg/dl. Eight healthy rabbits were included in the study as controls (Group 3). Blood samples for ANP analysis were obtained three months after administration of alloxan monohydrate.

The plasma ANP levels in moderately diabetic rabbits (328 ± 43 pg/ml) were significantly higher than those in normoglycemic (98.5 ± 20 pg/ml) and healthy (76.6 ± 18 pg/ml) controls ($p < 0.001$ for both). In addition, we found a significant correlation between plasma levels of glucose and levels of ANP ($r = 0.665$, $p < 0.001$).

Our data indicate that further experiments need to be performed to investigate what is responsible for the elevation of plasma ANP levels in diabetic rabbits. (Jpn Heart J 36: 789–795, 1995)

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ATRIAL natriuretic peptide (ANP) has been shown to be a hormone with potent natriuretic, diuretic and vasodilating properties, which plays an

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important role in the regulation of sodium and water.¹⁾ This hormone is typically released from the heart in response to atrial stretch.²⁾ However, many factors affecting regulation of ANP secretion have been suggested.^{3,4)} On the other hand, some diabetic states are associated with abnormalities in fluid and electrolyte balance.⁵⁻¹⁰⁾ Patients with insulin-dependent diabetes mellitus (IDDM) may have increased glomerular filtration rates at the onset or during the course of the disease.^{11,12)} There have been controversial reports regarding the plasma ANP levels in the diabetic state.^{5,7,8,13,14)} Ortola et al⁵⁾ reported evidence from animal (rat) studies that ANP may also play an important role in the increased glomerular filtration rate (GFR) of patients with IDDM. These authors noted increased plasma ANP levels and concomitant increases in GFR in rats made diabetic with streptozocin. However, Hebden et al,^{7,8)} Clark et al,¹³⁾ and Yano et al¹⁴⁾ were unable to demonstrate any major effect of streptozocin — induced diabetes mellitus on circulating ANP in the rat.

To investigate whether there is a relationship between hyperglycemia and plasma ANP levels, the present study was performed using 14 New Zealand rabbits made diabetic with alloxan monohydrate and 16 control animals.

MATERIALS AND METHODS

Thirty male New Zealand white rabbits weighing 2000 to 2600 g were used throughout the experiment. Animals were housed in metabolic cages for 7 days before the study to allow for adaptation at a constant room temperature of 21°C. Of these, 22 rabbits received a single intravenous injection of alloxan monohydrate (100 mg/kg, dissolved in saline at a concentration of 50 mg/ml). The diabetic state was examined 72 h later by quantitative determination of blood glucose levels of > 350 mg/dl. Diabetic animals were divided into two groups. Beginning on day 3, animals in group 1 ($n=14$) received a daily subcutaneous injection of 1 U of NPH insulin, an amount designed to maintain the blood glucose concentration (BGC) between 300 and 400 mg/dl (moderate hyperglycemia). Rabbits in group 2 ($n=8$) received 3.2 U of NPH insulin daily to maintain the (BGC) below 100 mg/dl (normoglycemic control). Rabbits in group 3 ($n=8$) were age- and weight-matched to those in groups 1 and 2, were not given alloxan monohydrate, and served as nondiabetic controls.

Three months after administration of alloxan monohydrate, animals were killed by decapitation and arterial blood samples for ANP determination were drawn into chilled heparinized syringes and into prechilled test tubes containing EDTA - 2 Na (1.5 mg/ml whole blood) and aprotinin (1000 kallikrein inactivator units per milliliter). The blood samples were then separated in a refrigerated centrifuge at 4000 rpm for 5 min. The plasma was removed, and

extracted within 48 h after collection according to the method of Lang et al¹⁵⁾ and passed over a C18 Sep-pak cartridge (Waters Associates; Inc Millipore Corp., Milford, MA, USA) prewashed with 4 ml of 60% acetonitrile in 0.1% trifluoroacetic acid, reconstituted with ANP radioimmunoassay buffer and stored at -40°C until assay with a commercially available radioimmunoassay kit (Incstar Corporation, Stillwater, MN, USA Cat. No: 22750) using human 125a-ANP. Interassay variability was 9% ($n = 5$) and intraassay variability 6% ($n = 5$).

Hemoglobin A1c was measured by column chromatography (Isolab Inc. Glyc-Affin GHb Code SG-6200, OH, USA). Before the hemoglobin assay, the erythrocytes were washed with 0.9% saline solution to remove the labile fraction, and each sample was run in duplicate. Normal hemoglobin A1c values for our laboratory are 3% to 6%. Serum glucose level was determined using the hexokinase method (Hitachi 717 autoanalyzer, Tokyo).

All results are expressed as means $\pm$ SE and comparisons were made with the unpaired Student's t-test; $p < 0.05$ was considered significant. Linear regression analysis was used for correlation.

RESULTS

Body weights and plasma glucose concentrations of the three groups are shown in Table I. Hyperglycemia was confirmed by BGC that were significantly higher than those in other groups (Groups 2 and 3). The BGC of diabetic rabbits treated with high-dose insulin (Group 2) did not differ from those in normal controls (Group 3).

Table I. Animal Groups

	Group 1	Group 2	Group 3
Number of animals	14	8	8
Body weight (g)	2233 $\pm$ 127	2281 $\pm$ 144	2331 $\pm$ 142
Plasma glucose (mg/dl)	356 $\pm$ 29.7	68.4 $\pm$ 27.3	82.9 $\pm$ 11.6

Group 1: Moderately hyperglycemic rabbits, Group 2: Normoglycemic diabetic rabbits, Group 3: Healthy control rabbits.

Table II. Mean Plasma ANP, HbA1c and Electrolyte Levels

Parameters	Group 1 ($n = 14$)	Group 2 ($n = 8$)	Group 3 ($n = 8$)
ANP (pg/ml)	328 $\pm$ 43 ^a	98.5 $\pm$ 20	76.6 $\pm$ 18
HbA1c (%)	4.68 $\pm$ 1.73 ^b	6.8 $\pm$ 2.7	3.7 $\pm$ 0.6 ^b
Sodium (mEq/l)	138.5 $\pm$ 14	139.6 $\pm$ 1.7	140.6 $\pm$ 1.6
Potassium (mEq/l)	4.3 $\pm$ 0.40	4.5 $\pm$ 0.36	4.5 $\pm$ 0.28

HbA1c = glycohemoglobin A1c; a = $p < 0.001$ as compared with group 2 and 3; b = $p < 0.001$ as compared with group 2.

Figure. Positive correlation between plasma ANP levels and plasma glucose concentration in rabbits with diabetes mellitus. ($p < 0.001$)

Body weights were not different between the groups at the beginning and upon completion of the study.

Plasma ANP, HbA1c and electrolyte levels in the three groups of rabbits are shown in Table II. A significant increase in plasma ANP was noticed in group 1 animals. HbA1c levels were slightly higher in group 2 animals than in the other groups. Serum sodium and potassium levels tended to be decreased in the moderately hyperglycemic rabbits, but the difference was not significant.

By regression analysis, a significant correlation was observed between plasma ANP levels and plasma glucose concentration ($r = 0.665$, $p < 0.001$; Figure).

DISCUSSION

The level of ANP found in healthy adult rabbit plasma, 76.6 ± 18.3 pg/ml, was comparable to that reported in the anesthetized rat of around 55 pg/ml⁽¹⁵⁾ and the anesthetized dog (60–120 pg/ml⁽¹⁶⁾), in healthy male rabbits (28–50 pg/ml⁽¹⁷⁾), and in anesthetized rabbits (around 30 pg/ml⁽¹⁸⁾).

There have been controversial reports regarding the plasma ANP levels found in the diabetic state.^{5,7,8,13,14} Although Hebden et al^(7,8) and Clark et al⁽¹³⁾ in the rat and Yano et al⁽¹⁴⁾ in the female non-obese diabetic mouse were unable to demonstrate any major effect of streptozocin (STZ)-induced diabetes mellitus on circulating ANP levels, Ortola et al⁽⁵⁾ reported that the plasma ANP levels doubled in diabetic rats which had been intravenously injected with STZ (60 mg/kg) 2 weeks prior to the experiment. The latter finding is consistent with the present study. The results of this study indicate that, in hyperglycemic diabetic

rabbits, plasma ANP levels were significantly elevated above normal values. The cause of these controversial reports may be due to species differences, or to the difference between the drug-induced and spontaneous animal models. To date, no study examining plasma ANP levels in the diabetic state has been made on rabbits. Ortola et al⁵⁾ speculated that moderate hyperglycemia, with its attendant chronic volume expansion, stimulates atrial ANP release, and that elevated plasma ANP levels might contribute to the hyperfiltration observed in early diabetes. However, the mechanism of the elevation of plasma ANP in diabetes mellitus is not known.¹³⁾ ANP secretion is known to increase in response to atrial stretch and is usually induced by volume expansion or redistribution of blood to the thorax.^{20,21)} In addition, hypertonicity may also be a stimulus to ANP secretion.²²⁾ ANP levels in diabetes may be influenced by a number of variables such as volume expansion, hypertonicity, and underlying vascular or renal disease. Clark et al¹³⁾ found no statistically significant correlation between plasma levels of insulin and levels of ANP. This suggests that insulin did not produce an increase in ANP levels. In this study, we found a significant correlation between plasma ANP levels and BGC. This finding is in agreement with the study of Ortola et al,⁵⁾ but not with the study of Clark et al.¹³⁾ Hyperglycemia might indirectly increase ANP levels. Glycosuria owing to hyperglycemia may lead to diuresis, which in turn causes extracellular dehydration.²³⁾ The reduction of extracellular volume could allow a more efficient cardiac ANP release, resulting in a higher sodium excretion.²⁴⁾ Also, in our study, the fact that serum sodium and plasma levels are lower in moderately hyperglycemic animals than both control groups, even though not significant, supports the idea mentioned above. In addition, Yano et al¹⁴⁾ have found an increase in right atrial ANP levels in diabetic mice as revealed by RIA and immunohistochemical staining of the atria. Todd et al²⁵⁾ have reported that most elevated levels of plasma ANP seem to be associated with decreased atrial content, which could be due to either an increase in atrial release or reduced plasma clearance. They speculated that the reduction in atrial tissue ANP may be associated with either a decrease in synthesis or an increase in mobilization and secretion. However, further studies will be required to elucidate the metabolic pathways, HbA1c and ANP correlation.

HbA1c levels were found to be lower in Group 1 than in Group 2. Since we used NPH insulin during the study, the hypoglycemic effect is approximately 12 hours. For the other half of the day, the rabbits were in a hyperglycemic condition. Consequently, hyperglycemia led to increases in HbA1c levels. The rapid fluctuations in blood glucose levels were more effective in promoting glycosylation of hemoglobin than the sustained hyperglycemia.²⁶⁾

In conclusion, the results of this study further demonstrated that plasma ANP levels were elevated in hyperglycemic rabbits with 3 months of alloxan-

diabetes compared with normoglycemic diabetic and nondiabetic control values. However, our data indicate that further experiments should be performed to investigate what is responsible for the elevation of resting plasma ANP levels in diabetic rabbits treated 3 months previously with alloxan monohydrate.

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