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Attenuated release of atrial natriuretic peptide and vasorelaxation in streptozotocin-induced diabetic rats

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The present study was aimed at investigating the atrial natiuretic peptide (ANP) and urinary responses to acute perturbations in fluid balance and the vascular function in diabetes mellitus (DM). DM was induced in rats by treatment with streptozotocin (50 mg/kg, i.p.). Ten weeks later, the plasma ANP concentration measured in the conscious state was significantly higher in DM group (27.5±3.9 pg/mL) than in the control (15.4±2.6 pg/mL), while the atrial tissue contents of ANP were lower. In response to acute extracellular volume expansion (VE), amounting up to 5% of body weight over 45 min, under thiopental anesthesia (50 mg/kg, i.p.), the magnitude of increase in plasma ANP was lower in the DM group than in the control (56.8±25.2 vs. 189.1±53.6% increases over the basal). Urinary sodium excretion during VE was also lower in the DM group. Acetylcholine-induced relaxation of the isolated aortic rings was attenuated in the DM group, which was partially restored by L-arginine-supplementation (2 g/L in drinking water). These results suggest that body fluid homeostasis and vascular functions are unfavorably altered in DM.

Key Words : *Diabetes mellitus, Atrial natriuretic peptide, Volume-expansion, Urinary sodium excretion, Endothelium-dependent vasorelaxation.*

INTRODUCTION

Diabetic patients are particularly prone to cardiovascular disorders (Tomlinson et al., 1992). Therefore, much attention has been paid in the etiology of cardiovascular consequences associated with diabetes mellitus (DM).

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Among the abnormalities in fluid balance and blood volume homeostasis seen in DM, an enhanced natriuresis and diuresis may be explained by elevations in plasma atrial natriuretic peptide (ANP) (Hebden et al., 1986). An abnormal urinary response to exogenous ANP (Patel and Zhang, 1989) may also, in part, account for the altered fluid balance. The ANP and urinary responses to acute perturbations in fluid balance in DM have not been established, however.

It has also been suggested that vascular endothelial function is altered in DM. The results representing responses of diabetic blood vessels to acetylcholine are conflicting, however. Decrease

(Oyama et al., 1986), increase (Bhardwaj and Moore, 1988) and no change (Head et al., 1987 ; Harris and MacLeod, 1988) have been reported. In addition, the precise mechanism for the altered vascular function remains to be solved.

The present study was aimed at investigating the ANP and urinary responses to acute perturbations in fluid balance and the vascular functions in DM. To induce DM in rats, streptozotocin (STZ) was used.

METHODS

Materials

Male rats (Sprague-Dawley), weighing 100-120 g, were used. They were injected with STZ (50 mg/kg, i.p.) and kept on regular rat chow and tap water for 10 weeks, being designated as [DM] group. [DM+Arg] group was not only treated with STZ, but also supplemented with L-arginine in the drinking water (2 g/L). A group of age-matched rats without STZ-treatment served as [control].

Plasma and atrial ANP

The rats were killed by decapitation and trunk blood was collected to determine plasma concentrations of ANP and renin in the conscious state. Both atria were removed to measure their ANP contents. The blood-sampling tubes contained a mixture of ethylenediaminetetraacetic acid (EDTA, 1 mg/mL of blood), phenylmethylsulfonyl fluoride (50 mmol/L), aprotinin (1,000 KIU/mL) and soybean trypsin inhibitor (SBTI, 50 BAEE/mL).

Effects of volume expansion on ANP release and urinary excretion

ANP and urinary responses to acute extracellular volume expansion (VE) were examined. Under thiopental (50 mg/kg, i.p.) anesthesia, the right femoral artery was cannulated to measure arterial blood pressure, and the vein to serve as an infusion route. A bladder catheter was implanted to collect urine samples.

A 30-60 min equilibration period was allowed to elapse until urine collection started. Urine was collected every 15 min by flushing the bladder with 1 mL of distilled water followed by 1 mL of air injected through the bladder catheter. Basal urine data (volume and sodium excretion) were obtained by averaging two or three period values before VE was begun.

VE was induced by intravenous infusion of isotonic saline (0.9% NaCl) over 45 min. Total volume infused amounted up to 5% of body weight. Blood samples were taken before and after VE.

Radioimmunoassay of ANP and renin

Blood samples were centrifuged at 4℃, and the plasma was kept at -70℃ until analyzed. The plasma was thawed, extracted with Sep-Pak C18 cartridges (Waters Associates ; Milford, MA), and lyophilized. The right and left atria were separately homogenized in 1.0 N acetic acid, heated for 10 min in a boiling water bath, centrifuged, and the supernatant was lyophilized.

The lyophilized samples were reconstituted with Tris-acetate buffer (0.1 mol/L, pH 7.4, containing 0.2% neomycin, 10 mmol/L EDTA, 50 BAEE/mL SBTI, 0.02% sodium azide, 200 KIU/mL aprotinin and 1% bovine serum albumin). Concentrations of ANP in the aliquots were determined using a radioimmunoassay kit (Research & Diagnostic Antibodies ; Berkeley, CA). The determined values were corrected with the extraction ratio (68.5± 0.4%).

Plasma renin concentration (PRC) was also determined by radioimmunoassay using unextracted plasma samples as described previously (Cho et al., 1987).

Isolated vascular preparations

Upon decapitation, the thoracic aorta was removed. Its rings 5-mm long each were prepared in ice-cold saline. Each ring was mounted in a muscle bath by sliding it over two parallel stainless-steel hooks. The lower hook was fixed to the bottom of the bath, and the upper was connected to the isometric transducer (Grass FT03), to record the changes in isometric tension.

The bath contained physiological salt solution at 37±0.5℃, and was continuously bubbled with 95% O_2-5% CO_2. Baseline load placed on the ring was 2.0 g. The composition (in mmol/L) of the solution was NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 11.5. Drugs used were phenylephrine hydrochloride (Sigma), acetylcholine chloride (Sigma) and sodium nitroprusside (Abbott).

Statistics

Results are expressed as means±SE. The statistical significance was determined using two-way ANOVA and paired or nonpaired *t*-test. Calculated p values were adjusted for multiple comparisons using the Bonferroni correction factor, where applicable.

RESULTS

Plasma and atrial ANP

Blood glucose concentrations measured upon decapitation were significantly higher in the DM group (417±27 mg/dL, n=5) than in the control (103±5 mg/dL, n=7).

Fig. 1 shows the basal plasma ANP and PRC in the conscious state. ANP was significantly higher in the DM group (27.5±3.9 pg/mL) than in the control (15.4±2.6 pg/mL), while PRC was lower {24.2 ±1.3 vs. 51.6±3.0 ngAI/(mL·h)}. Both the right and left atrial tissue contents of ANP were significantly lower in the DM group (Fig. 2).

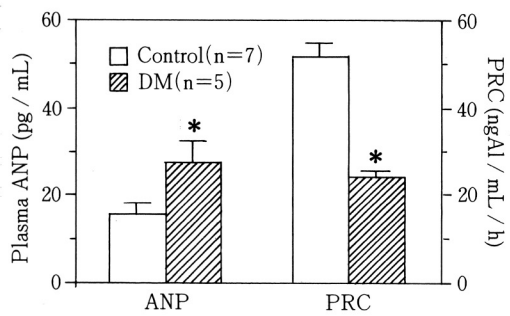

Fig. 1. Plasma atrial natriuretic peptide (ANP) and renin concentrations (PRC) in the diabetic (DM) and control groups. n=number of rats in each group. *p<0.05, compared with the control.

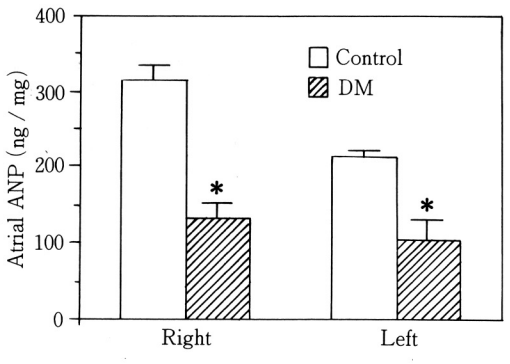

Fig. 2. Right and left atrial tissue contents of ANP. Legends as in Fig. 1. *p<0.05, compared with the control.

ANP response to volume expansion

Fig. 3 shows the plasma ANP before and after VE. The plasma ANP significantly increased following VE in both DM and control groups, the magnitude of which was lower in the former than in the latter (56.8±25.2 vs. 189.1±53.6% increases over the basal).

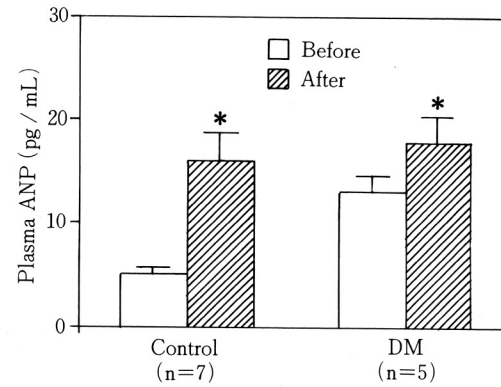

Fig. 3. Plasma ANP concentrations before and after volume expansion. Legends as in Fig. 1. *p<0.05, compared with the before value in each group.

Urinary responses to volume expansion

Basal urine volume was significantly higher in the DM group than in the control, while sodium excretion was not different. During VE, the volume was not different between the two groups, but the sodium excretion was significantly lower in the DM group (Fig. 4).

Responses of isolated aortic rings

The maximal tension of the isolated aortic ring, attained by phenylephrine (3.5×10^{-6} mol/L), was lower in the DM group (1.18±0.23 g) than in the control (2.00±0.21 g). L-Arginine-supplemented group showed a partial restoration of the maximal

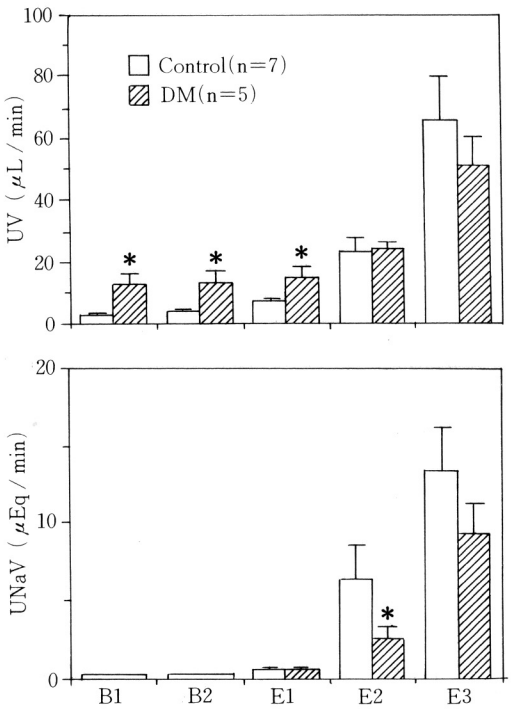

Fig. 4. Urinary volume and sodium excretion before and during volume expansion. B1 and B2 represent two consecutive periods before VE started, each period being 15 min. E1-E3 denote three periods during VE. During VE, total sodium excretion was significantly lower in the DM group compared with the control ($p<0.05$). *$p<0.05$, compared with the control in the corresponding period.

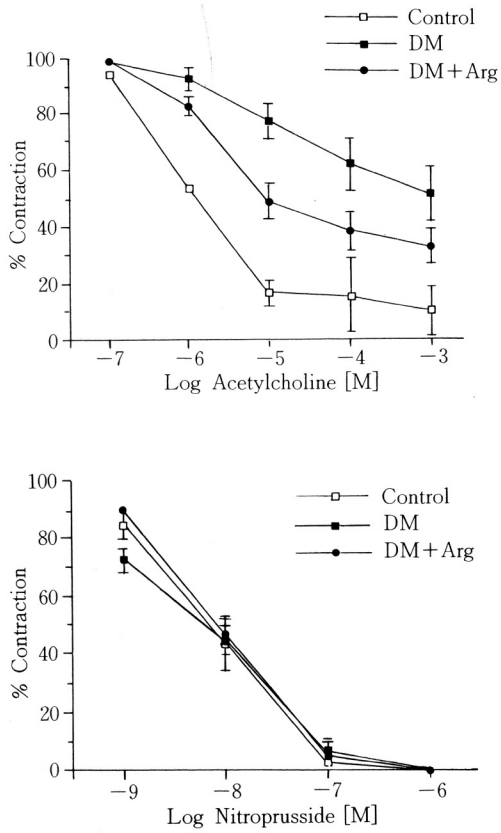

Fig. 5. Relaxation responses to acetylcholine and nitroprusside of the isolated thoracic aorta precontracted with phenylephrine. DM : diabetic group. DM+Arg : diabetic group supplemented with L-arginine in the drinking water. Control : non-diabetic control group. Each point represents mean±SE from 6-10 experiments.

tension (1.66±0.17 g).

Fig. 5 shows the vasorelaxation responses to acetylcholine and nitroprusside, when the maximal tension was induced and maintained by phenylephrine. Acetylcholine elicited a dose-dependent relaxation, the magnitude of which was attenuated in the DM group. L-Arginine-supplemented group showed a partial restoration of the relaxation. In comparison, the magnitude of relaxation induced by nitroprusside did not differ among the groups.

DISCUSSION

STZ-induced DM rats showed increases in basal plasma ANP, as has been found by many previous investigators (Black and Lee, 1989 ; Hebden et al., 1989 ; Matsubara et al., 1990 ; Todd et al., 1990). In comparison, the atrial tissue contents of ANP were reduced in the DM rats.

It has been found that the tissue ANP content may be determined by its secretory rate (Takayanagi et al., 1985). Therefore, the higher plasma ANP associated with the reduced tissue content suggests an increased basal release. The previous studies (Hebden et al., 1989 ; Todd et al., 1990) showing a decreased atrial granularity and an elevated plasma ANP following STZ-treatment also support such a possibility.

The mechanism underlying the increased ANP release is unclear. Although an unaltered right atrial

pressure in STZ-treated rats despite the increased plasma ANP has been found (Hebden et al., 1989), an increase in circulating volume may not be ruled out (vide infra). A direct effect of insulin lack on the secretory process of ANP may also be speculated, as for the renin release (Katayama and Lee, 1985).

In response to acute extracellular VE, the magnitude of the ANP release was reduced in the DM group. The attenuation could be attributable to reduced tissue ANP stores. Interestingly, however, the plasma ANP values following VE did not differ between the DM and control groups, implying that a maximal response to a given stimulus is essentially the same regardless of the initial pathophysiological state.

The urinary sodium excretion in response to VE was also reduced in the DM group. It has been found that the number of renal cortical ANP receptors decreases in DM (Benigni et al., 1990). It may be hypothesized that DM primarily induces functional changes in the ANP system, which, in turn, results in alterations of renal function, through a receptor down-regulation.

Since sodium is a primary determinant of the body fluid volume, its decreased excretion may contribute to an increase of extracellular fluid volume in DM. The lower PRC in the DM group may be attributed to either the increased ANP, or an increased plasma volume, or both.

On the other hand, diabetic vessels showed a reduced relaxation in response to acetylcholine, while their response to nitroprusside did not change. These results would indicate a selective impairment of endothelium-dependent relaxation. Much evidence has been accumulated for an impaired endothelium-dependent vasodilation in DM (Oyama et al., 1986 ; Pieper and Gross, 1988). Furthermore, the present study also showed that diabetic vessels were with a reduced contractility in response to phenylephrine.

The mechanisms underlying the attenuated responses are not completely understood. The partially restored relaxation and contraction by the L-arginine-supplementation suggests that the impaired response could be prevented by L-arginine. Among others, a decreased production of nitric oxide may be responsible.

creased ANP and urinary responses in DM, which are potentially important with regard to the hemodynamics and blood volume homeostasis. The altered vascular functions may contribute to deteriorations of cardiovascular homeostasis in DM.

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