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Research Article

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Elevated Plasma Atrial Natriuretic Peptide Levels in Diabetic Rats

Potential Mediator of Hyperfiltration

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Abstract

Infusion of atrial natriuretic peptide (ANP) increases the glomerular filtration rate (GFR), and ANP is released from cardiac myocytes in response to extracellular fluid volume expansion. Since diabetes mellitus is associated with glomerular hyperfiltration and volume expansion, we investigated the relationship between ANP and GFR in diabetic rats given insulin to achieve stable moderate hyperglycemia or normoglycemia. At 2 wk after induction of diabetes, moderately hyperglycemic diabetic rats exhibited elevations of plasma ANP levels averaging 281±28 pg/ml vs. 158±15 pg/ml in normoglycemic diabetic rats. In hyperglycemic rats, the GFR was also elevated on average to 2.24±0.28 ml/min as compared with 1.71±0.13 ml/min in normoglycemic diabetic rats. To test further the relationship between ANP and GFR in diabetes, moderately hyperglycemic diabetic rats were infused either with a specific ANP antiserum or with nonimmune serum. The infusion of specific ANP antiserum uniformly reduced the GFR on average from 2.38±0.1 ml/min to 1.60±0.1 ml/min, whereas the infusion of nonimmune serum was without effect. It is concluded that elevated endogenous ANP levels contribute to the hyperfiltration observed in early diabetes in the rat.

Introduction

Early stages of diabetes mellitus are characterized by elevations of the glomerular filtration rate (GFR)[1] and renal plasma flow (RPF) rate (1, 2). Experimental studies in the rat made diabetic with streptozotocin indicate that diabetes induces a state of intrarenal vasodilatation, with elevation of the single nephron glomerular capillary plasma flow rate (Q_A). Because the reduction in afferent arteriolar resistance is proportionately greater than that in efferent arteriolar resistance, the mean glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) tends to rise (3–5). Thus, glomerular hyperperfusion and hypertension contribute to the observed single nephron hyperfiltration in diabetes. Despite intensive investigation, however, the mechanisms that underlie the hyperfiltration of diabetes remain elusive. One contributing factor may be extracellular fluid volume expansion, which has been reported in clinical (6) and experimental (3, 7) diabetic states associated with moderate hyperglycemia.

Atrial natriuretic peptide (ANP) is a hormone released by atrial myocytes in response to acute (8) and chronic (9) extracellular volume expansion. In addition to the marked stimulation of renal sodium excretion, intravenous infusions of atrial extracts (10) or synthetic atrial natriuretic peptides (11) significantly augment the GFR. Micropuncture studies in the rat have demonstrated that this increase in GFR during ANP infusion is due to a reduction in afferent but not efferent arteriolar resistance, and thus an increase in $\bar{P}_{GC}$ (11).

The present study was designed to test the hypothesis that the extracellular fluid volume expansion associated with moderate hyperglycemia in the diabetic rat stimulates cardiac ANP release and that elevated circulating levels of ANP serve to augment GFR, thereby contributing to glomerular hyperfiltration commonly observed in the diabetic state.

Methods

Studies were undertaken in 58 male Sprague–Dawley rats weighing 200–220 g. Of these, 44 rats received a single intravenous injection of streptozotocin (60 mg/kg body wt). The diabetic state was confirmed 48 h later by quantitative determination of blood glucose levels of > 400 mg/dl. 10 rats with sustained blood glucose concentrations between 100 and 300 mg/dl were excluded from the study. All rats were allowed free access to standard rat chow (Rodent Lab Chow 5001; Ralston Purina Co., St. Louis, MO).

The remaining 34 diabetic rats were divided into two groups. Beginning on day 3, animals in group 1B (n = 16) received a daily subcutaneous injection of 1 U insulin (Ultralente Novo; Novo Industri, Copenhagen, Denmark), an amount designed to maintain the blood glucose concentration between 300 and 400 mg/dl (moderate hyperglycemia). Rats in group 1C (n = 10) received 3.2 U of insulin daily to maintain the blood glucose concentration below 100 mg/dl (normoglycemia). Rats in group 1A (n = 10) were age- and weight-matched to those in groups 1B and 1C, were not given streptozotocin, and served as nondiabetic controls. Blood glucose concentration was determined thrice weekly in all animals, and values for each rat are given as the mean of all determinations during the 2-wk study period.

Of the 34 diabetic rats, 8 were studied under anesthesia, and the other 26 were killed by decapitation. Of the 14 control rats, 4 were studied under anesthesia and 10, sacrificed by decapitation as detailed below.

Plasma ANP levels. The rats were killed by decapitation on day 15, and trunk blood was collected in iced tubes containing 15 mg EDTA,

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1. *Abbreviations used in this paper:* ANP, atrial natriuretic peptides; GFR, glomerular filtration rate; iANP, immunoreactive ANP; PAH, para-aminohippurate; $\bar{P}_{GC}$, mean glomerular capillary hydraulic pressure; Q_A, glomerular capillary plasma flow rate; RPF, renal plasma flow rate; SNGFR, single nephron GFR.

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aprotinin (1,500 kallikrein-inactivator units), and 10 N-α-benzoyl-L-arginine ethyl ester units of soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO) for subsequent extraction and determination of plasma immunoreactive ANP (iANP) levels.

GFR. To determine the GFR, four rats from each group (designated groups 2A, 2B, and 2C, each $n = 4$) were not killed as described above, but underwent surgery and inulin clearance determinations on day 15. For this purpose rats were anesthetized with Inactin (100 mg/kg body wt i.p.) and placed on a temperature-regulated table. After tracheostomy, a polyethylene (PE-50) catheter was inserted in the left femoral artery for blood sampling and to monitor the arterial blood pressure. 500 μl of blood for iANP and blood glucose determinations were drawn at the beginning of the experiment, before infusion of plasma or inulin. This blood was replaced volume for volume with plasma obtained from correspondingly glycemic diabetic rats. Catheters were also placed in the left femoral, right jugular veins, and the left ureter. To replace plasma losses associated with anesthesia and surgery (12), isoncotic rat plasma obtained from diabetic rats or from normal rats, equivalent in volume to 1% of body wt, was infused over 50 min, followed by a sustained infusion of 0.6 ml/h throughout the remainder of the experiment. Inulin (7%) and para-aminohippurate (PAH) (0.8%) in normal saline was infused at a rate of 1.2 ml/h. After 30 min equilibration, urine was collected from the left ureteral catheter during two 10-min periods, and 210 μl of blood was taken at the midpoint of each collection to quantify inulin, sodium, and potassium concentrations. At the end of the experiment, 2 ml blood was collected for determination of glycosylated hemoglobin with a commercially available assay kit (Glyco-gel; Pierce Chemical Co., Rockford, IL) (13).

ANP antiserum infusions. To test the potential role of endogenous ANP in diabetic hyperfiltration, the following protocol was designed to block the effects of endogenous ANP. 14 moderately hyperglycemic diabetic rats were infused with a high-affinity sheep antiserum raised against ANP or with a nonimmune sheep serum (Sigma Chemical Co.). Rats underwent surgery for GFR determination as described above on days 14 to 16 after streptozotocin injection. After baseline measurements of GFR and urinary sodium excretion, a continuous intravenous infusion of the specific ANP antiserum (group 3B) or of nonimmune sheep serum (group 4B) was begun. 1:60,000 dilution of the sheep ANP antiserum is known to bind ~ 50% of radiolabeled ANP at a concentration of 50–100 pM. Therefore, the antiserum was diluted 1:60 with normal saline and infused at a rate of 0.105 ml/h. This rate of infusion was calculated to achieve a plasma antiserum concentration that could bind 50% of ANP every minute, assuming a plasma volume of 10 ml and a steady state plasma ANP concentration of ~ 75 pM (230 pg/ml). The nonimmune serum was infused at the same rate. Measurements of GFR and urinary sodium excretion were then repeated after a 90-min equilibration period. The blood glucose concentration was determined at the beginning and the end of the study.

The antiserum used in this study has a very high affinity for the carboxy terminus of ANP, as evidenced by the fact that rat ANP 1–28 and 4–28 and human ANP 1–28 showed 50% binding at an antiserum dilution of 1:60,000, all with equal affinity. By contrast, ANP 3–27 and 5–27, which are lacking the carboxy-terminal tyrosine residue, showed < 5% cross-reactivity with this antiserum.

Analytical. Blood glucose concentrations were determined with a reflectance meter (Miles Ames Div., Miles Laboratories, Inc., Elkhart, IN). The concentration of inulin in plasma and urine was measured by the anthrone method (14). PAH concentration was determined by a colorimetric technique (15). Plasma and urine sodium and potassium concentrations were measured by flame photometry.

The plasma ANP was extracted as described by Lang et al. (8). Plasma was diluted with 4% acetic acid (1:3 vol/vol) and was passed over a C_{18} Sep Pak cartridge (Water Associates, Millipore Corp., Milford, MA) prewashed with 10 ml methanol, washed with 10 ml distilled water, eluted with 90% ethanol and 0.4% acetic acid, and evaporated to dryness. The dried eluate was reconstituted with ANP-radioimmunoassay (RIA) buffer, and all eluates were stored at −40°C. For each sample, two different dilutions of the plasma extract were assayed in duplicate for iANP using a commercially available RIA kit (Peninsula Laboratories, Inc., Belmont, CA), and the mean value is reported. The recovery of extracted ANP, as determined by addition of unlabeled ANP to plasma, was 75±0.5% ($n = 7$). The intra- and interassay coefficients of variations were 8.1% ($n = 7$) and 11.0% ($n = 7$), respectively.

Statistical. Data are presented as means±SEM. The data were analyzed using one-way analysis of variance, and the significance, determined by the Scheffe's method for two comparisons (16). The Student's paired t test was used for analysis between groups 3B and 4B. Regression analysis was performed using standard formulas. The statistical significance was defined as $P < 0.05$.

Results

Systemic parameters. Whole animal data for groups 1A, 1B, and 1C are summarized in Table I. In group 1B, moderate hyperglycemia was confirmed by blood glucose levels that were significantly higher than those in nondiabetic rats (group 1A). The blood glucose concentrations of diabetic rats treated with high-dose insulin (group 1C) did not differ from those in controls. Blood glucose concentrations were stable during the 2-wk study period.

Body weights were not different among groups at the beginning and the completion of the study. Despite similar body weights, renal hypertrophy was apparent in the moderately hyperglycemic group 1B rats, whereas kidney weight in normoglycemic diabetic rats was no higher on average than in nondiabetic control rats.

Individual values for plasma iANP levels in the three groups of rats are shown in Fig. 1. Plasma iANP levels were elevated in moderately hyperglycemic group 1B rats, which averaged 281±28 pg/ml ($P < 0.005$) as compared with the nondiabetic group 1A rats (137±11 pg/ml). By contrast, normoglycemic diabetic rats (group 1C) exhibited values for plasma iANP (158±15 pg/ml) no different from those in nondiabetic controls. Thus, moderate hyperglycemia in the rat is characterized by elevated plasma iANP levels, which may be normalized with strict glycemic control. By regression analysis, a significant correlation was observed between plasma ANP levels and blood glucose concentration ($r = 0.64$, $P < 0.001$).

Renal hemodynamic data. Whole animal and hemodynamic data for rats that underwent clearance measurements are shown in Table II. Again, body weight did not differ among the groups. Group 2B rats were moderately hyperglycemic, whereas blood glucose levels in group 2C were maintained at levels comparable to those in nondiabetic rats (Group 2A). Maintenance of chronic moderate hyperglycemia in group 2B was further confirmed by measurement of glycosylated hemo-

Table I. Blood Glucose, Body, and Kidney Weights

Group	Blood glucose	BW	KW
	mg/dl	*g*	*g*
1A ($n = 10$)	89±3	311±4	2.3±0.1
1B ($n = 16$)	326±13*	298±5	2.9±0.1*
1C ($n = 10$)	85±4	313±6	2.3±0.1

Values are means±SEM. BW, body weight; KW, kidney weight.
* $P < 0.01$ vs. group 3A.

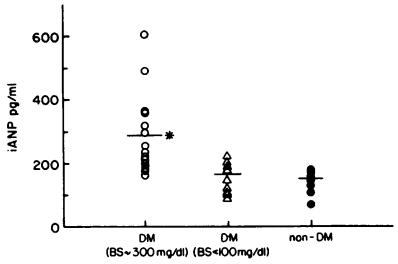

Figure 1. Plasma iANP in individual moderately hyperglycemic diabetic rats (DM, blood sugar [BS] ~ 300 mg/dl), normoglycemic diabetic rats (DM, BS < 100 mg/dl) and nondiabetic control rats (non-DM). * $P < 0.005$ vs. DM (BS < 100 mg/dl) and non-DM.

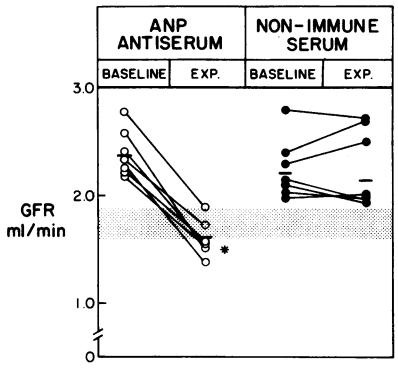

Figure 2. GFR in moderately hyperglycemic rats in the baseline period and during infusion of either ANP antiserum (*left*) or nonimmune serum (*right*). Shaded band denotes mean value±SD for nondiabetic control rats in this laboratory. Horizontal bars denote mean values. * $P < 0.001$ vs. baseline.

globin. Glycosylated hemoglobin levels in group 2B rats were significantly higher at 19.9±0.2%, on average, than those of 3.5±0.2% in control rats ($P < 0.01$). In the normoglycemic diabetic rats (group 2C), glycosylated hemoglobin levels of 5.2±0.9% did not differ from values in control rats. At the time of surgery, mean arterial pressure did not differ among groups. In accord with previous studies (3–5), moderately hyperglycemic rats (group 2B) exhibited elevated values for GFR as compared with values in nondiabetic controls ($P < 0.05$). By contrast, hyperfiltration was not observed in the diabetic rats in which blood glucose was kept in the normal range by higher doses of insulin (group 2C). Plasma sodium and potassium concentrations were similar in all groups. Plasma iANP levels measured in arterial blood from anesthetized rats undergoing clearance determinations were again elevated in the moderately hyperglycemic diabetic rats (group 2B) as compared with those in nondiabetic control rats ($P < 0.05$). Plasma iANP levels in normoglycemic diabetic rats (group 2C) were equivalent to those in nondiabetic rats. Plasma iANP levels in these anesthetized rats were generally lower than those determined in blood samples from conscious rats, in the previous protocol. These differences are felt to reflect the known effect of barbiturate anesthesia in suppressing plasma iANP levels (17).

ANP antiserum infusions. Individual values for GFR before and during infusion of the ANP antiserum or the nonimmune serum are shown in Fig. 2. As in the previous protocol, hyperfiltration was observed in these moderately hyperglycemic rats during the baseline period (2.38±0.09 ml/min in group 3B, 2.20±0.20 ml/min in group 4B). Blood glucose concentrations measured at the initial and end points of the experiment did not change (308±13 vs. 301±18 mg/dl). Mean arterial pressure averaged 113±3 and 115±3 mmHg in groups 3B and 4B, respectively, in the baseline period, and remained unaltered at 111±4 mmHg during ANP antiserum infusion and at 112±4 mmHg during nonimmune serum infusion. However, during the ANP antiserum infusion, values for GFR uniformly declined to levels in the low normal range (1.6±0.06 ml/min, $P < 0.001$ vs. baseline). By contrast, infusion of the nonimmune serum had no significant effect on the GFR, which was stable at 2.12±0.08 ml/min. The RPF as estimated by the PAH clearance also decreased significantly, from 6.69±0.48 ml/min in baseline to 5.21±0.50 ml/min ($P < 0.005$) during the ANP antiserum infusion, whereas in the rats treated with nonimmune serum, RPF did not change (6.07±0.61–6.30±0.44 ml/min). Further evidence that ANP antiserum infusion blocked the effects of endogenous ANP was provided by measurements of urinary sodium excretion, which were reduced on average from 0.83±0.17 to 0.45±0.15 μEq/min during ANP antiserum infusion ($P < 0.005$). In group 4B, urinary sodium excretion in the baseline period was 1.14±0.3 μEq/min and remained unchanged at 1.28±0.24 μEq/min during infusion of nonimmune serum. The reduction in urinary sodium excretion in rats infused with ANP antiserum exceeded that which resulted from the concurrent fall in GFR, in that fractional excretion of sodium fell in each rat (from 0.29±0.05% to 0.20±0.05%, $P < 0.005$), an effect not seen with nonimmune serum.

Discussion

Despite the high incidence of end-stage renal disease in patients with diabetes mellitus, the early stages of this disease are characterized by glomerular hyperfiltration, with values for GFR averaging ~ 40% higher than those in age-matched, nondiabetic controls (1, 2, 18–20). These elevated filtration rates are usually accompanied by increments in renal plasma flow (19) and by renal hypertrophy (2, 20). Hyperfiltration and renal hypertrophy similar to those in humans have been docu-

Table II. Systemic Hemodynamic and Renal Parameters at 2 wk of Diabetes

Group	BG	BW	$\overline{AP}$	GFR	P[Na]	P[K]	Plasma iANP
	mg/dl	*g*	*mmHg*	*ml/min*	*mEq/liter*	*mEq/liter*	*pg/ml*
2A (n = 4)	88±6	307±11	114±4	1.71±0.10	146±2	3.9±0.0	34.8±3.3
2B (n = 4)	357±21*	290±11	118±3	2.24±0.14*	141±1	4.2±0.1	79.9±11.1*
2C (n = 4)	79±6	317±7	118±3	1.71±0.13	148±3	3.7±0.1	34.2±3.5

Values are means±SEM. BG, blood glucose; BW, body weight; $\overline{AP}$, mean arterial pressure; P[Na], plasma sodium concentration; P[K], plasma potassium concentration. * $P < 0.05$ vs. group 2A.

mented in several experimental models. Hostetter and co-workers (3) found that maintenance of moderate hyperglycemia in streptozotocin–diabetic rats was associated with 40% increases in whole kidney and single nephron GFR (SNGFR). The present study confirms previous reports that moderately hyperglycemic diabetic rats exhibit glomerular hyperfiltration and renal hypertrophy, and that both glomerular hyperfiltration and renal hypertrophy are prevented in diabetic rats kept normoglycemic with high doses of insulin (21–22).

Recent evidence suggests that the initiation and progression of diabetic renal injury may relate to the increases in glomerular capillary pressures and flows rather than to metabolic factors per se (23). Clinically, those patients with the highest GFR at the time diabetes is diagnosed are most likely to progress to persistent proteinuria or overt diabetic nephropathy (24). In diabetic rats, Zatz and co-workers (4) demonstrated that limitation of SNGFR, $\bar{P}_{GC}$, and Q_A with dietary protein restriction retards the development of albuminuria and glomerular injury, even in the absence of any improvement in metabolic control as assessed by blood glucose or glycosylated hemoglobin levels. Of the hemodynamic determinants of single nephron hyperfiltration, the critical role of glomerular capillary hypertension was highlighted in subsequent studies using the angiotensin I converting enzyme inhibitor enalapril (5). In this normotensive model, a modest reduction of systemic blood pressure was accompanied by normalization of $\bar{P}_{GC}$, without any change in SNGFR or Q_A, and by long-term protection against glomerular injury (5). Taken together, these observations strongly suggest that renal hemodynamics exert a pivotal influence on the initiation and progression of diabetic glomerulopathy.

The mechanisms whereby diabetes mellitus induces glomerular hyperfiltration remain undefined. Initial institution of insulin therapy reduces GFR and RPF in type I diabetics (25), GFR declines with conversion from conventional to intensive insulin therapy (26), and acute insulin infusion reduces GFR in diabetic patients (27) and diabetic rats (28).

One factor which may figure prominently in diabetic hyperfiltration is plasma volume expansion. Elevated plasma volumes have been documented in moderately controlled type I diabetics (6) as well as in several models of diabetes in the rat (3, 7), including rats prepared exactly as in groups 1B and 2B of the present study (unpublished observations). Associated abnormalities in diabetes include suppression of plasma renin activity (7) and plasma aldosterone concentration (29). These features of the diabetic state, together with systemic vasodilatation, are strikingly similar to the known effects of ANP, a hormone which is released from cardiac atria in response to plasma volume expansion (8, 9). Infusion of ANP results in vasodilatation, augmentation of $\bar{P}_{GC}$ and GFR (11), and in suppression of plasma renin activity (30) and adrenal aldosterone release (31).

In the present study, plasma concentrations of endogenous ANP were clearly elevated in the moderately hyperglycemic diabetic rats, as they are in other states associated with plasma volume expansion, including dietary sodium excess (9) and mineralocorticoid excess (32). It seems likely that normalization of plasma ANP levels in the normoglycemic diabetic rats in the present study reflected the more normal volume status associated with tight metabolic control.

The findings in this study are consistent with the hypothesis that moderate hyperglycemia, with its attendant chronic plasma volume expansion, stimulates atrial ANP release, and that elevated plasma ANP levels may contribute to the hyperfiltration observed in diabetes. The availability of ANP specific antiserum allowed us to further test this association between elevation of ANP and hyperfiltration in diabetes. Blockade of endogenous ANP was confirmed by reductions in urinary sodium excretion and fractional excretion of sodium, parameters not similarly affected by nonimmune serum. The observation that infusion of the specific ANP antiserum uniformly reduced the GFR values to the normal range lends strong support to the hypothesis that elevation of circulating ANP is a potentially important mediator of the hyperfiltration of diabetes. Furthermore, the reduction of GFR during ANP antiserum infusion demonstrates for the first time that enhanced endogenous ANP release can raise the GFR. An increase of RPF is an important component of hyperfiltration in diabetes. This elevation of RPF observed in moderately hyperglycemic diabetic rats was also reversed by the infusion of the specific ANP antiserum. This reduction in RPF would also contribute to the observed fall in GFR and denotes an important action of ANP in the regulation of the renal circulation.

In summary, this study demonstrates that moderate hyperglycemia in diabetic rats is associated with an elevation in circulating ANP levels and in GFR, and that hyperfiltration in these moderately hyperglycemic rats is reversed by infusion of an ANP-specific antiserum. These findings are interpreted to indicate that ANP mediates, at least in part, the hyperfiltration state in diabetic rats.

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