

Original Contributions

Functional Implications of Decreased Renal Cortical Atrial Natriuretic Peptide Binding in Experimental Diabetes

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Glomerular hyperfiltration in streptozotocin-induced diabetes mellitus in rats may be mediated by atrial natriuretic peptide (ANP). We wanted to evaluate plasma levels of ANP and plasma volume in relation to renal ANP receptor density and affinity in rats 6 weeks after induction of diabetes. Plasma levels of immunoreactive ANP were significantly higher in hyperglycemic diabetic (75.2 ± 8.3 pg/ml) than in control animals (34.7 ± 8.1 pg/ml; $p < 0.01$). Administration of insulin to keep diabetic rats normoglycemic normalized plasma levels of immunoreactive ANP (30.5 ± 5.2 pg/ml). In contrast, plasma volume did not show significant differences among the groups (hyperglycemic diabetes, 46.6 ± 3.8 ; normoglycemic diabetes, 42.4 ± 3.2 ; controls, 43.2 ± 2.0 ml/kg body wt). No correlation was found between plasma levels of immunoreactive ANP and plasma volume. By autoradiography a significant reduction in the number of renal cortical ANP receptors was observed in hyperglycemic diabetic rats as compared with controls. At variance, ANP receptor affinity did not change either in the cortex or in the medulla in hyperglycemic diabetics in comparison with control animals. The pathophysiological implication of cortical ANP receptor down-regulation was underscored by the blunted response of glomerular filtration rate to ANP infusion in diabetic animals as compared with controls. These results indicate that in hyperglycemic diabetic rats: 1) the plasma volume is numerically higher without reaching a statistically significant value and does not correlate with the elevated plasma levels of ANP; 2) the marked rise in plasma concentration of ANP is associated with a significant reduction in the density of ANP binding sites in the renal cortex, without measurable changes in medullary receptors; and 3) the blunted glomerular hemodynamic response to ANP infusion underscores the importance of down-regulation of cortical ANP receptors in modulating the sustained hyperfiltration of early diabetes. (*Circulation Research* 1990;66:1453–1460)

Early diabetes mellitus in experimental animals^{1–4} and humans^{5–7} is associated with an increase in glomerular filtration rate (GFR). Micropuncture studies in rats made diabetic with streptozotocin indicate that glomerular hyperfiltration results from the concomitant elevation in glomerular plasma flow rate and mean glomerular capillary hydraulic pressure after the reduction in afferent arteriolar resistance, which is proportionately greater than that in efferent arteriolar resistance.^{2,8,9} Several studies tried to identify the factor responsible for glomerular hyperfiltration in human as well as in experimental diabetes. Thus, indomethacin reduces single-nephron GFR and glo-

merular capillary hydraulic pressure in hyperfiltering diabetic rats, suggesting that intrarenal prostaglandins might contribute to the maintenance of elevated GFR in experimental diabetes.¹⁰ A reduced glomerular angiotensin II receptor density in the presence of a suppressed renin-angiotensin axis has also been reported as one of the contributing factors to the decreased renal arteriolar resistance and hyperfiltration in early diabetes.¹¹

Recently, it has been proposed that hyperfiltration in experimental diabetes may be mediated by atrial natriuretic peptide (ANP), a hormone released by cardiac atria¹² that reduces afferent but not efferent arteriolar resistance,¹³ thus increasing GFR. The evidence that ANP may participate in the hyperfiltration of diabetes rests on the observation of Ortola et al⁴ that plasma levels of ANP are elevated in rats made diabetic with streptozotocin and that the infusion of a specific ANP antiserum in these animals normalizes GFR.

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The mechanism of ANP increase in diabetic animals has not been established yet, but the most likely explanation seems to be plasma volume expansion.

The present study was designed to clarify: 1) the possibility that elevated circulating levels of ANP are the result of the plasma volume expansion, 2) the effect of the possible variations in plasma volume on the density and affinity of ANP binding sites in the kidney, and 3) the functional response of the kidney to the infusion of ANP.

Materials and Methods

Experimental Design

All studies were performed with male Wistar rats (Charles River Breeding Laboratories, Calco, Italy) weighing 275–300 g. Animals were kept on an ad libitum diet of standard rat chow and had free access to tap water. Diabetes was induced by a single injection of streptozotocin (60 mg/kg body wt i.v.) dissolved in citrate buffer (pH 4.2) into the tail vein after overnight fasting as described previously.³ Age- and weight-matched control rats received a sham injection of vehicle. Within 3 days after streptozotocin administration, the diabetic state was confirmed by the development of polyuria and polydipsia and by quantitative determination of blood glucose concentration >400 mg/dl. Animals with blood glucose levels between 100 and 300 mg/dl were excluded from the study. Five days after streptozotocin injection, diabetic rats were randomly assigned to either of two groups. Rats in group 1 ($n=6$) received a daily subcutaneous injection of a very long-acting heat-treated insulin¹⁴ (pH 5.5; Ultralente MC, NOVO Industri, Copenhagen, Denmark) individually adjusted (mean, 0.9 IU insulin) to maintain blood glucose concentration between 300 and 400 mg/dl (hyperglycemia). Rats in group 2 ($n=6$) were given 5–10 IU/day insulin (mean 6.5 IU) to achieve strict metabolic control defined as normal rate of increase in body weight, blood glucose level below 100 mg/dl (normoglycemia), and absence of polyuria. Rats in group 3 ($n=6$) were nondiabetic (control). Blood glucose concentrations were measured three times a week by tail blood samples with a reflectance meter (Ames, Miles Laboratories, Elkhart, Indiana). Body weight was determined once a week. The presence of glomerular hyperfiltration in the early stage of experimental diabetes mellitus was established by resting the animals for renal clearance under anesthesia 6 weeks after streptozotocin injection. Glomerular filtration rate and renal plasma flow (RPF) were determined by the clearance of inulin and *para*-aminohippurate (PAH), respectively, as described below.

To verify the association of increased GFR and changes in plasma levels of immunoreactive ANP (i-ANP) and the possible contribution of extracellular fluid volume expansion to the release of ANP by atrial myocytes, three additional groups of rats were studied. All rats were age- and weight-matched to those used for clearance studies. Group 4 ($n=6$)

consisted of diabetic rats maintained hyperglycemic by insulin; group 5 ($n=6$) consisted of diabetic rats maintained normoglycemic by insulin; group 6 ($n=6$) consisted of nondiabetic control rats. Plasma volume was determined 6 weeks after streptozotocin or vehicle administration by the dilution principle with radioiodinated serum albumin,¹⁵ as described below. The animals were then killed by decapitation and trunk blood was collected for i-ANP measurements.

Furthermore, to assess the relation between plasma i-ANP levels and the renal ANP receptor distribution and density, kidneys were removed and immediately frozen before quantitative *in vitro* autoradiography was performed.

To investigate whether changes in renal ANP receptors may be a compensatory phenomenon for modulating the increase in GFR, hyperglycemic diabetic (group 7, $n=6$), normoglycemic diabetic (group 8, $n=6$), and control (group 9, $n=6$) rats were infused with exogenous ANP, and renal function was assessed before and after exposure to the peptide, as described below.

Renal Clearance Studies

Rats were anesthetized by injection of thiopental sodium (50 mg/kg body wt i.p.). Surgical preparation consisted of tracheostomy, cannulation of femoral artery and vein, and placement of a large-bore polyethylene tube in the bladder through a small suprapubic incision. A heated table maintained body temperature at 37°C. Arterial blood pressure was continuously monitored throughout the experiment directly from the femoral artery by a polyethylene tube (PE-50) connected to a pressure transducer and a carrier amplifier (Battaglia Rangoni, Bologna, Italy). GFR and RPF were measured by the clearance of inulin (E. Merck, Darmstadt, FRG) and PAH (Sigma Chemical, St. Louis, Missouri), respectively. Inulin (7.5%) and PAH (0.2%) in normal saline were infused in the femoral vein as a priming load, followed by constant infusion of the same solution via a syringe pump at 1.5 ml/hr. A 40-minute equilibration period was allowed after surgery and initiation of infusion to attain constant plasma levels of inulin and PAH. Clearance periods, 30 minutes each, were performed in triplicate. At the midpoint of each period, 100- μ l blood samples were obtained from the femoral artery and plasma was separated by centrifugation. Urine samples were collected from the bladder in preweighed vessels. Plasma and urine inulin and PAH were determined by methods previously described,^{16,17} modified for microliter samples.

Measurements of Plasma Volume

Plasma volume was measured as the iodinated I 131 albumin distribution volume. For this purpose, iodinated I 131 human serum albumin (Sari-1, Sorin Biomedica, Saluggia, Italy) was injected (0.4 μ Ci i.v.) through the tail vein. To yield better accuracy and to eliminate overestimation of plasma volume,¹⁵ 10 and 20 minutes later blood samples (100 μ l) were col-

lected, and plasma was obtained and counted. The net counts of samples were plotted on semilogarithmic paper against time and, by extrapolation, the "zero time" counts were derived and used for calculations.

Measurement of Plasma i-ANP

Plasma concentrations of i-ANP were determined; the rats were killed by decapitation, and trunk blood was collected in iced polyethylene tubes containing EDTA (1 mg/ml) and aprotinin (1,000 kallikrein inhibitor units/ml). Plasma was separated by centrifugation at 2,500g at 4° C for 15 minutes and stored at -80° C until extraction, which was performed according to the method of Lang et al.¹⁸ Briefly, 3 ml plasma containing 1,200 cpm of synthetic rat 1-28 ¹²⁵I-ANP (Amersham International, Buckinghamshire, UK) was mixed with 9 ml of 4% acetic acid and centrifuged for 10 minutes at 1,700g. The supernatant was collected and applied on a C₁₈ Sep Pak cartridge (Water Associates, Millipore, Milford, Massachusetts). The column was then washed with 5 ml distilled water and eluted with 86% ethanol in 4% acetic acid; the latter fraction was collected and evaporated to dryness. The dried residue was reconstituted in ANP radioimmunoassay buffer.

One hundred microliters of serial dilutions of each plasma extract was assayed in duplicate for i-ANP by using a commercially available radioimmunoassay kit (Peninsula Laboratories, Belmont, California). Good linearity was observed between concentrations of i-ANP from 1 to 128 pg/tube. The lowest detectable concentration that could be measured with 95% confidence was 8 pg/tube. i-ANP levels for all samples were corrected for the recovery, which averaged 75% (range 70–80%) as determined by the addition of radiolabeled ANP to rat plasma before extraction.

The intra-assay and interassay coefficients of variation were 9% and 12%, respectively.

Autoradiography of ¹²⁵I-ANP Binding Sites

After rat decapitation, kidneys were immediately removed, cleaned of adhering fat, and frozen in liquid nitrogen. Sections (16 μm) were cut on a cryostat (Ames) at -15° C, thaw-mounted on gelatin-coated glass slides, and stored at -20° C. For autoradiographic studies, the sections were incubated in Hanks' balanced salt solution (pH 7.4) containing bovine serum albumin 0.2%, HEPES 10 mM, glucose 5.5 mM, and bacitracin 0.1%. The rat 1-28 ¹²⁵I-ANP (Amersham) and the unlabeled rat 1-28 ANP (Bachem Feinchemikalien, Bubendorf, Switzerland) were used for radioreceptor studies. For binding inhibition studies, serial sections were incubated with 50 pM ¹²⁵I-ANP (specific activity, 1,950–2,000 Ci/mM) in the absence or presence of a range of concentrations (10⁻¹⁰–10⁻⁷ M) of unlabeled ANP. After 90 minutes of incubation in an ice-water bath, slides were rapidly washed three times for 1 minute each in Hanks' balanced salt solution (pH 7.4) at 4° C and then dried under a stream of cool air, placed in

x-ray cassettes, and exposed to Hyperfilm-³H (Amersham) for 10 days at -80° C.

Quantification of ¹²⁵I-ANP binding was performed with the use of a set of ¹²⁵I tissue standards included in each cassette. Standards were prepared by mixing kidney homogenate with known quantities of ¹²⁵I. Each of six standards was frozen, and sections (16 μm thick) were cut on a cryostat at -15° C and thaw-mounted on gelatin-coated glass slides. Adjacent sections were also taken for determination of total radioactivity. After exposure, the films were developed in Kodak D-19 (Eastman Kodak, Rochester, New York) for 4 minutes and fixed in Unifix Kodak for 5 minutes. Optical densities of autoradiograms were determined with a RAS-1000 image analyzer (Amersham) and converted to femtomoles of ¹²⁵I-ANP/mm²¹⁹ by a regression analysis that compared the optical densities of the sections with those of the standards.

Binding parameters (B_{\max} and K_d) of renal ANP receptors from control and diabetic rats were analyzed with the ALLFIT program,²⁰ and B_{\max} and K_d were calculated from ALLFIT estimated parameters. B_{\max} was expressed in femtomoles per square millimeter. To assess the protein concentration per surface unit area of kidney sections in diabetic and control rats, we measured protein content by a modified Lowry method,²¹ and the area by image analyzer.

To describe the distribution of ANP receptors along the renal sections, we used the kidney subdivision proposed by the Renal Commission of the International Union of Physiological Sciences.²²

Renal Response to ANP Infusion

Six hyperglycemic, six normoglycemic diabetic rats, and six control animals underwent surgery for GFR and RPF determination, as described above, 6 weeks after streptozotocin injection. On completion of the baseline period (three 10-minute clearances), synthetic rat ANP (1-28, Bachem Feinchemikalien), dissolved in phosphate-buffered saline (0.01 M, pH 7.4), was infused at the rate of 1 μg/kg/min during a 35-minute experimental period. Clearances were not determined until the dead space in the kidney and bladder was cleared. For this purpose, clearances (10 minutes each) were evaluated starting 5 minutes after the beginning of ANP infusion. A midpoint blood sample for each clearance was obtained. Urine and plasma samples were analyzed for inulin and PAH as described above.

Statistical Analysis

Data are presented as mean ± SD. Data were analyzed by one-way analysis of variance. Duncan's multiple-range test, which permits multiple testing among treatment means, was then used. Correlation between plasma volume and plasma i-ANP levels was performed by linear regression analysis. Plasma i-ANP values were logarithmically transformed before they

TABLE 1. Whole Animal and Renal Hemodynamic Parameters at 6 Weeks After Diabetes Induction or Vehicle Administration

	BG (mg/dl)	BW (g)	KW (g)	MAP (mm Hg)	GFR (ml/min/100 g)	RPF (ml/min/100 g)
Group 1	365.5±19.4*	382.6±7.3	1.30±0.04†	116.0±4.0	1.23±0.04*	2.90±0.36*
Group 2	88.5±6.4	396.1±10.5	1.07±0.04	115.5±3.9	0.86±0.09	2.20±0.12
Group 3	90.5±5.1	399.3±11.7	1.08±0.05	116.5±2.7	0.85±0.08	2.19±0.23

Values are mean±SD (n=6). BG, blood glucose; BW, body weight; KW, kidney weight; MAP, mean arterial blood pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; group 1, hyperglycemic diabetic rats; group 2, normoglycemic diabetic rats; group 3, control rats.

* $p<0.01$ vs. groups 2 and 3.

† $p<0.05$ vs. groups 2 and 3.

were plotted because they were not normally distributed. The effect of ANP infusion on GFR and RPF in normal and diabetic rats and the comparison of renal response with ANP infusion between and within normal and diabetic rats were tested by using the analysis of variance (mixed design) with Tukey's method for multiple comparison.²³ Statistical significance was defined as $p<0.05$.

Results

Metabolic and Functional Determinants of the Model

Table 1 shows whole animal and hemodynamic data in rats that underwent renal function studies. Blood glucose levels were significantly ($p<0.01$) higher in hyperglycemic diabetic rats (group 1) than in control rats. The blood glucose concentrations of normoglycemic diabetic rats (group 2) did not differ from those of controls (group 3). Blood glucose concentrations were stable during the study period. Before clearance studies, hyperglycemic, normoglycemic diabetic, and control animals had comparable body weight (Table 1).

Hyperglycemic diabetic rats exhibited significantly ($p<0.01$) higher values of GFR than controls. In contrast, insulin administered to diabetic rats to maintain their blood glucose concentration within the normal range determined a complete normalization of GFR. The trend of RPF measurements paralleled that of GFR. Thus, RPF was significantly ($p<0.01$) elevated in hyperglycemic diabetic rats with respect to controls. In contrast, RPF in rats undergoing strict glycemic control was comparable with that measured in control rats (Table 1).

When the animals were killed, kidney weight was significantly ($p<0.05$) increased in hyperglycemic diabetic as compared with normoglycemic diabetic or with control rats.

Plasma Levels of i-ANP and Plasma Volume Measurements

At the time of plasma i-ANP measurement, body weight did not differ among the groups (group 4, 393.0±8.2; group 5, 391.5±20.3; group 6, 393.5±16.9 g). Hyperglycemic diabetic animals had blood glucose concentrations of 358.0±34.5 mg/dl; normoglycemic diabetic and control rats had blood glucose

concentrations of 100±8.8 and 96.8±6.6 mg/dl, respectively.

Plasma levels of i-ANP were significantly ($p<0.01$) higher in hyperglycemic diabetic rats (75.2±8.3 pg/ml) than in control rats (34.7±8.1 pg/ml). The administration of insulin to diabetic rats to maintain their blood glucose concentration within the normal range resulted in a normalization of plasma levels of i-ANP (30.5±5.2 pg/ml).

Plasma volume measurements did not show significant ($p=0.070$) differences among the groups, the values being 46.6±3.8, 42.3±3.1, and 43.2±1.9 ml/kg body wt in hyperglycemic diabetic, normoglycemic diabetic, and control rats, respectively.

A good correlation coefficient was found between plasma levels of i-ANP and blood glucose concentration ($r=0.94$, $p<0.001$). No correlation was observed ($r=0.04$) between plasma levels of i-ANP and plasma volume (Figure 1).

Localization of ANP Receptors and Binding Characteristics

The distribution of total ¹²⁵I-ANP binding in the kidney of control rats is shown in Figure 2. Computer analysis of the autoradiographic images revealed a punctate pattern of high-density binding in the cortex overlying glomeruli. Glomeruli at all levels in the

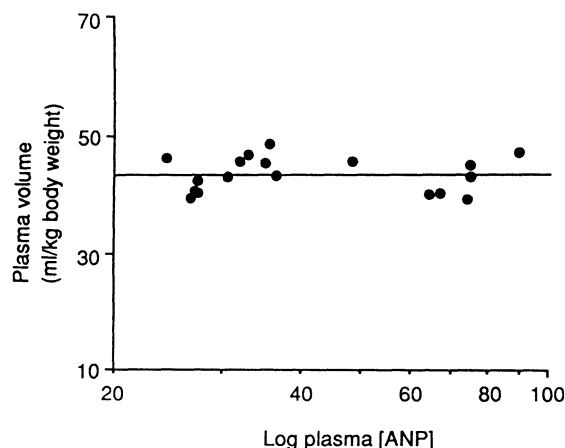


FIGURE 1. Linear regression analysis of plasma volume versus logarithmic values of plasma immunoreactive atrial natriuretic peptide (ANP) levels in diabetic (hyperglycemic and normoglycemic) and control rats ($r=0.04$, NS).

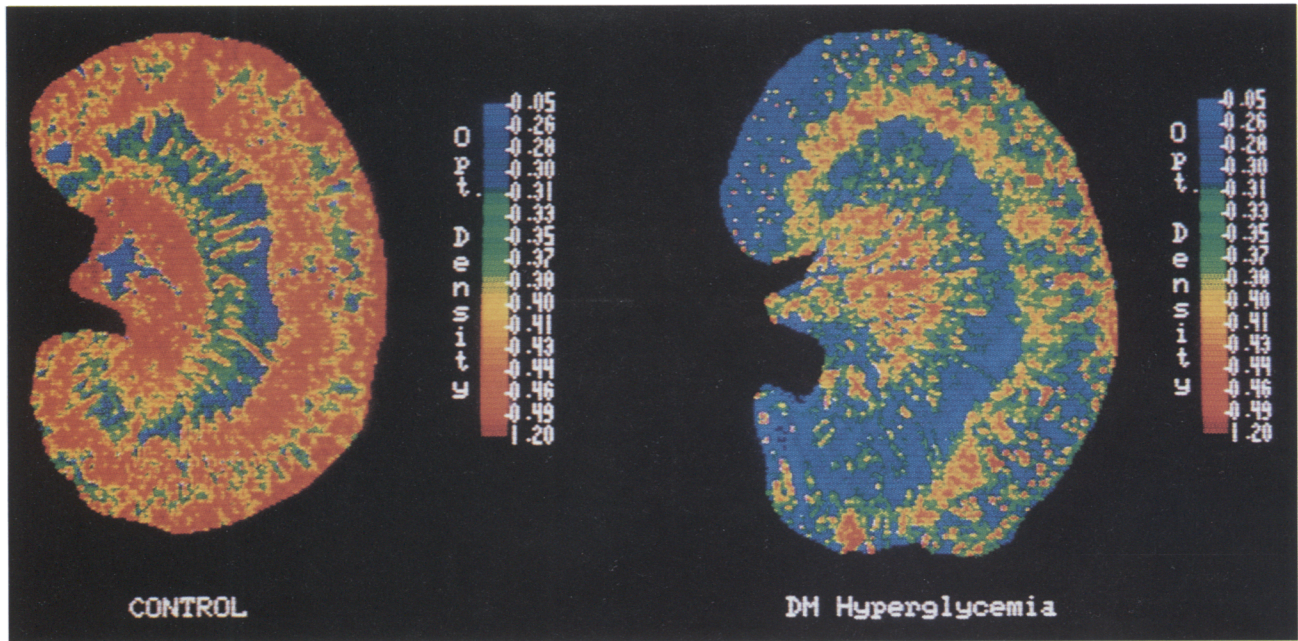


FIGURE 2. Computer-generated pseudocolor image of autoradiograms of total ^{125}I -labeled atrial natriuretic peptide (ANP) (1-28) binding to rat kidney. The optical density scale reports high densities of label (red) and low densities of label (blue). Left panel: Section from control rat. Right panel: Section from hyperglycemic diabetic (DM) rat. Note the marked difference in the total binding in the cortex between these sections. The difference in ^{125}I -ANP total binding in the inner medulla does not result in a significant difference in specific binding, since nonspecific binding is lower in diabetic than in nondiabetic kidney sections.

cortex were heavily labeled. In the inner stripe of the outer medulla a moderate density of binding was observed in longitudinal bands that overlaid vasa recta bundles. A heavily labeled area was found in the outer stripe of the outer medulla. However, at variance with binding in the cortex and in the inner stripe of the outer medulla, this was not displaced by 1×10^{-7} M unlabeled ANP, indicating that the binding in this zone was not specific. A high density of diffuse binding was found in the inner medulla, according to the observations of Koseki et al.²⁴

The density of binding in the cortex in hyperglycemic diabetic rats was reduced as compared with control rats (Figure 2), whereas no difference was observed between normoglycemic diabetic and control rats. The density of labeled ANP binding in the outer medulla was not different between the diabetic and control rats. Total ^{125}I -ANP binding in the inner medulla was apparently lower in hyperglycemic dia-

betic than in control rats (Figure 2). Because nonspecific binding was also lower in hyperglycemic diabetic than in control animals, no difference in specific binding in the inner medulla was observed between the two groups.

Binding parameters for cortex and outer and inner medulla were determined separately on serial sections incubated with a range of concentrations of unlabeled ANP. Specific binding to the cortex and outer and inner medulla was saturable and completely inhibited in the presence of 1×10^{-7} M unlabeled ANP. A single class of high-affinity receptors for ^{125}I -ANP was observed in each kidney zone. Binding parameters of each individual zone are reported in Table 2. In the group of hyperglycemic diabetic rats a significant ($p < 0.05$) decrease in maximum binding sites (B_{\max}) was detected in the cortex (0.064 ± 0.013 fmol/mm²) in comparison with normoglycemic diabetic (0.087 ± 0.008 fmol/mm²) or with

TABLE 2. ^{125}I -ANP Receptor Binding Density and Affinity in Kidney at 6 Weeks After Diabetes Induction or Vehicle Administration

	Cortex		Inner stripe of outer medulla		Inner medulla	
	B_{\max} (fmol/mm ²)	K_d (nM)	B_{\max} (fmol/mm ²)	K_d (nM)	B_{\max} (fmol/mm ²)	K_d (nM)
Group 4	$0.064 \pm 0.013^*$	0.270 ± 0.104	0.095 ± 0.013	0.346 ± 0.051	0.160 ± 0.040	0.397 ± 0.100
Group 5	0.087 ± 0.008	0.382 ± 0.077	0.080 ± 0.009	0.324 ± 0.048	0.155 ± 0.018	0.367 ± 0.003
Group 6	0.090 ± 0.016	0.329 ± 0.069	0.086 ± 0.015	0.322 ± 0.046	0.152 ± 0.021	0.340 ± 0.034

Values are mean \pm SD ($n = 6$). B_{\max} , maximum binding sites; K_d , binding dissociation constant; group 4, hyperglycemic diabetic rats; group 5, normoglycemic diabetic rats; group 6, control rats.

* $p < 0.05$ vs. group 5; $p < 0.01$ vs. group 6.

control rats (0.090 ± 0.016 fmol/mm²). It is well known that diabetes is associated with kidney volume expansion; thus, the observed reduced binding in terms of femtomoles per square millimeter might reflect dilution of the receptors. To exclude this possibility, we measured protein concentration per unit surface area and found it comparable in diabetic and control rats (hyperglycemic diabetic, 4.40 ± 0.73 ; normoglycemic diabetic, 3.78 ± 0.39 ; control, 4.27 ± 0.5 μ g/mm²). The binding dissociation constant (K_d) was not significantly different between diabetic and control rats, independent of the blood concentration of ANP.

As shown in Table 2, analysis of the autoradiograms did not reveal changes in the maximum number of binding sites and in the dissociation constant of the ANP receptor in the inner and outer medulla among the three groups of animals.

Renal Response to ANP Infusion

Diabetic rats were hyperglycemic (group 7, 341 ± 25 mg/dl) or normoglycemic (group 8, 95 ± 5 mg/dl) when the renal response to ANP infusion was evaluated. The control animal blood glucose concentration was in the normal range (92 ± 5 mg/dl).

Hyperglycemic diabetic rats showed significantly ($p < 0.01$) higher values of GFR (1.19 ± 0.01 ml/min/100 g) than normoglycemic diabetic rats (0.85 ± 0.07 ml/min/100 g) or nondiabetic controls (0.81 ± 0.06 ml/min/100 g). The infusion of synthetic ANP significantly ($p < 0.01$) increased GFR both in diabetic and in control animals in comparison with preinfusion values (hyperglycemic diabetics: preinfusion, 1.19 ± 0.01 ; postinfusion, 1.28 ± 0.02 ml/min/100 g; normoglycemic diabetics: preinfusion, 0.85 ± 0.06 ; postinfusion, 1.54 ± 0.07 ml/min/100 g; controls: preinfusion, 0.81 ± 0.05 ; postinfusion, 1.49 ± 0.10 ml/min/100 g). However, GFR did not increase to the same extent in the three groups of rats. Indeed, the estimated percent increase of average GFR of the three 10-minute ANP infusion periods from preinfusion values was significantly ($p < 0.01$) lower in hyperglycemic diabetic ($8.1 \pm 0.9\%$) than in normoglycemic diabetic or in control animals (normoglycemic diabetics, $81.2 \pm 14.5\%$; controls, $83.2 \pm 1.9\%$).

RPF values, measured before ANP infusion, were significantly ($p < 0.01$) more elevated in hyperglycemic diabetic rats than in diabetic rats rendered euglycemic by insulin or in nondiabetic controls. The infusion of synthetic ANP resulted in a significant increase of RPF in all three groups (hyperglycemic diabetics: preinfusion, 2.88 ± 0.10 , postinfusion, 3.03 ± 0.03 ml/min/100 g, $p < 0.05$; normoglycemic diabetics: preinfusion, 2.20 ± 0.09 ; postinfusion, 3.00 ± 0.14 ml/min/100 g, $p < 0.01$; controls: preinfusion, 2.13 ± 0.07 ; postinfusion, 3.14 ± 0.11 ml/min/100 g, $p < 0.01$).

As observed for GFR, the estimated percent increase of RPF from preinfusion values of the average of three 10-minute periods was significantly ($p < 0.01$) lower in hyperglycemic diabetic ($5.1 \pm$

3.3%) than in normoglycemic diabetic ($36.6 \pm 5.8\%$) or in control rats ($47.3 \pm 0.6\%$).

Discussion

It has been documented by Ortola et al⁴ that in moderately hyperglycemic diabetic rats glomerular hyperfiltration and renal hypertrophy are associated with increased levels of ANP. Both glomerular hyperfiltration and renal hypertrophy were prevented in diabetic animals kept normoglycemic with insulin, which also prevented the increase in plasma levels of ANP. This observation suggests that ANP is one of the mediators of hyperfiltration in experimental diabetes. The present experiments confirm that hyperglycemic diabetic rats have increased levels of ANP, which parallel the increase in GFR, and show that insulin treatment reduces both ANP levels and GFR.

To clarify the possible pathophysiological role of ANP in experimental diabetes, we analyzed plasma levels of ANP and plasma volume in relation to ANP receptor density and affinity. The rationale for these studies rests on the observation that a dynamic relation has been documented between plasma levels of ANP, plasma volume, and ANP receptor number.²⁵⁻²⁸

The results show that plasma volume is numerically higher in hyperglycemic diabetic versus normal rats or rats rendered euglycemic by insulin, but the difference does not reach statistically significant values. These findings apparently would not support the attractive possibility that the high plasma levels of ANP in rats with experimental diabetes and hyperglycemia are due to an extracellular fluid volume expansion. However, because plasma volume provides only an indirect assessment of atrial pressure and the difference between diabetics and controls may still have a "biological significance," the above-mentioned possibility cannot be ruled out on the basis of the present results. Data available so far on measurements of extracellular volume in experimental and human diabetes are contradictory. High blood volume has been documented in both alloxan-diabetic²⁹ and streptozotocin-diabetic² rats. In particular, Hostetter et al,² using the streptozotocin model, have shown that blood volume was significantly higher in hyperglycemic rats than in normal rats. By contrast, Jensen et al⁸ have been unable to document differences in plasma volume between streptozotocin-diabetic rats (independent of the metabolic control) and normal rats. Controversial results of plasma volume measurements have also been reported in humans affected by diabetes mellitus. Some studies^{30,31} have documented that plasma volume is higher in diabetics than in matched controls. In contrast, others have found comparable values for plasma volume in diabetics and controls.^{32,33}

At variance with blood volume, we have found that plasma levels of ANP significantly correlate with blood glucose levels, suggesting that the degree of metabolic control may be the signal for atrial cells to release ANP. However, further studies are necessary to establish a possible causal relation between the

metabolic control and the plasma levels of ANP in experimental and possibly human diabetes.

Independent of the mechanism responsible for the elevated ANP levels in hyperglycemic diabetic rats, we have shown that the increased plasma levels of ANP are associated with a significant reduction in the number of ANP receptors measured in the cortex, the renal medullary receptors being unaltered. The present study also documents that insulin treatment in diabetic rats, besides normalizing ANP levels, also normalizes the cortical density of ANP binding sites. At variance with the density, ANP receptor affinity does not change either in the cortex or in the medulla in hyperglycemic diabetic rats as compared with control rats. A down-regulation of ANP receptors appears the most obvious explanation for the negative correlation between plasma levels of ANP and its glomerular binding sites. The present findings are consistent with the notion that the circulating level of peptide hormones may directly regulate target tissue receptor number, and changes in plasma concentrations may negatively correlate with the density of specific receptors.³⁴ This phenomenon applies to circulating ANP, whose levels are known to determine receptor density at target tissue.^{25,27,35}

The finding that, at variance with glomerular receptors, the medullary ANP receptor density is unchanged in hyperglycemic diabetic rats merits a comment. To our knowledge, this is the first report that simultaneously analyzes ANP receptor density and affinity both at cortical and medullary levels in the presence of high plasma ANP levels. Finding a significant decrease in renal glomerular ANP binding sites without measurable changes in medullary binding sites possibly indicates that glomerular receptors are more accessible to circulating ANP than tubular receptors. So far, the cellular localization of ANP receptors has been well characterized at the glomerular level,³⁶ while no precise information is available for the distinct distribution of ANP receptor in the tubules.³⁷ It is possible that glomerular endothelial cell receptors are the most prone to down-regulation by high circulating levels of ANP.

The pathophysiological implication of ANP receptors' down-regulation in experimental diabetes was explored in the present study by infusing synthetic ANP to control and diabetic rats. In all groups of animals the infusion of ANP significantly increased GFR as compared with basal values, but the percent changes over the basal GFR values were significantly higher in control and normoglycemic diabetic than in hyperglycemic diabetic rats. Similarly, the increases in RPF over the basal values in response to ANP infusion were higher in normoglycemic diabetic and control rats as compared with hyperglycemic diabetic rats. The blunted hemodynamic response to ANP in hyperglycemic diabetic animals appears likely to be the consequence of the reduced number of cortical binding sites and may represent a compensatory mechanism to modulate the deleterious effects of

excessive hyperfiltration in diabetes. This modulatory pattern may have a major protective role against faster deteriorating renal function in diabetes since early hyperfiltration and hyperperfusion can cause glomerular structural abnormalities^{38,39} and ultimately lead to a progressive glomerulosclerosis.

In summary, the present study shows that in hyperglycemic diabetic rats: 1) the plasma levels of ANP are significantly increased in comparison with control animals; 2) the plasma volume is numerically but not statistically higher than in control rats and does not correlate with the elevated plasma ANP levels; 3) the marked rise in plasma ANP concentration is associated with a significant reduction in the density of ANP binding sites measured in the renal cortex, without measurable changes in medullary receptors; and 4) glomerular hemodynamic response to the infusion of synthetic ANP is blunted in hyperglycemic diabetic as compared with normoglycemic diabetic and control rats.

These findings may be interpreted to indicate that the mechanism of glomerular hyperfiltration in experimental diabetes is the result of a complex interaction between hormonal and metabolic factors and to underscore the possible role of down-regulation of cortical ANP receptors in modulating the sustained hyperfiltration.

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