Elevated Atrial Natriuretic Peptides and Early Renal Failure in Type 2 Diabetic Goto-Kakizaki Rats

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The present investigation was designed to determine if atrial natriuretic peptides (ANPs) are increased in a spontaneous model of non-obese type 2 diabetes, the Goto-Kakizaki (GK) rat. Four peptide hormones originating from the ANP prohormone were increased twofold ($P < .05$) to sixfold ($P < .01$) in the circulation of GK rats compared with nondiabetic Wistar rats from which the GK colony was originally derived. Thus, ANP, long-acting natriuretic peptide (LANP), vessel dilator, and kaliuretic peptide were (mean ± SE) 497 ± 78, 1,285 ± 105, 457 ± 45, and 385 ± 87 pg/mL in GK rats, versus 78 ± 23, 542 ± 77, 137 ± 26, and 134 ± 33 pg/mL, respectively, in Wistar rats. In evaluating the cause of the increased ANPs, the blood volume of GK rats (16.2 ± 0.4 mL) was significantly ($P < .01$) increased compared with Wistar rats (9.5 ± 0.3 mL). The ventricles of GK rats were not dilated when examined by transthoracic echocardiography, but the venous system was markedly distended. GK rats had a 48% to 79% decrease in renal function (ie, increased serum creatinine and blood urea nitrogen [BUN]) compared with Wistar rats. These results indicate that circulating ANPs are increased in the GK spontaneously diabetic rat secondary to (1) increased blood volume, which leads to increased synthesis and release of ANPs, and (2) renal failure, which results in a delayed metabolic processing of these peptides. The early combined increases of the four atrial peptides collectively may contribute to the hyperfiltration that occurs in early diabetes mellitus.

ONE OF THE atrial natriuretic peptides, atrial natriuretic factor, also called atrial natriuretic peptide (ANP), has been found to be increased in the plasma of streptozotocin (STZ)-induced diabetic rats examined at 2 to 12 weeks after induction of diabetes,[1-6] where it is thought to contribute to the hyperfiltration observed in these rats.[1,4,6] The ANP hormonal system consists of a 126–amino acid (aa) prohormone synthesized within myocytes of the heart and stored in granules within the heart for release into the circulation.[7-9] This hormonal system contains several peptides from the same 126-aa prohormone with blood pressure–lowering, natriuretic, diuretic, and/or kaliuretic properties[10-12] (Fig 1). Thus, peptides consisting of aa 1 to 30 (ie, long-acting natriuretic peptide [LANP]), aa 31 to 67 (vessel dilator), aa 79 to 98 (kaliuretic peptide), and aa 99 to 126 (ANP) of the ANP prohormone each have blood pressure–lowering, diuretic, natriuretic, and/or kaliuretic properties in both humans[11,12] and animals.[10,13] The ANP prohormone is partially proteolytically cleaved within the heart, and a 98-aa amino terminus and 28-aa carboxyl terminus (ie, ANP) of this prohormone are released into the circulation.[14-17] In the circulation, vessel dilator, LANP, and kaliuretic peptide circulate as distinct entities after proteolytic cleavage from the rest of the amino terminus of the ANP prohormone by proteases[11,18-20] (Fig 1).

Vessel dilator, LANP, and ANP bind to specific receptors[21-23] and subsequently enhance the activity of the particulate form of guanylate cyclase (EC 4.6.1.2) as part of their mechanism(s) of action.[13,24] The enhancement of guanylate cyclase activity by each of the respective ANPs increases the intracellular messenger cyclic guanosine monophosphate (GMP),[13,24,25] which causes vasodilation. Vessel dilator and LANP also inhibit renal Na^+,K^+-adenosine triphosphatase (ATPase) as part of their natriuretic mechanism(s) of action.[26,27] In contradistinction, ANP does not have any effect on renal Na^+,K^+-ATPase.[26-29] Vessel dilator and LANP each enhance prostaglandin E_2 synthesis, which appears to be the mediator of inhibition of Na^+,K^+-ATPase by these peptides.[26,27] Vessel dilator, LANP, kaliuretic peptide, and ANP are released simultaneously with central hypervolemia.[20,30] These peptide hormones are also released simultaneously in vitro from isolated perfused atria by atrial distention.[31]

Since STZ is a potent stimulator of guanylate cyclase[32] and since ANP appears to cause hyperfiltration by enhancing renal guanylate cyclase,[24,25] there has been controversy in the STZ model of diabetes as to whether the observed increase in ANP is due to STZ increasing ANP, which in turn increases cyclic GMP, or to the diabetic state.[1-6] To answer this question, the present investigation was designed to evaluate each of the above-described ANPs in a spontaneous rat model of type 2 non-obese diabetes mellitus, the GK rat.[33,34] When vessel dilator, LANP, kaliuretic peptide, and ANP were found to be markedly elevated within the circulation of the GK rat, the mechanism(s) responsible for the increase in these ANP hormones was investigated by determining if hypervolemia and/or decreased renal function caused the increase.

MATERIALS AND METHODS

Non-obese Spontaneous Type 2 Diabetes Rat Model

The GK model of non-obese type 2 diabetes mellitus was produced by selective brother-sister inbreeding of 18 nondiabetic Wistar rats that had glucose intolerance on oral glucose tolerance tests.[33,34] All offspring of GK rats are similarly affected by mild hyperglycemia within the first 2 weeks after birth.[35] In 1992, we initiated a colony of GK rats in Tampa from the F36 generation of the original colony in Japan,[35] kindly provided by Drs Suzuki and Toyota of Tokoku University (Sendai, Japan). In characterizing the colony housed at the Tampa Veterans Administration Hospital, we have found that these GK rats have a defect in synthesizing or releasing functional *chiro*-inositol–containing inosi-

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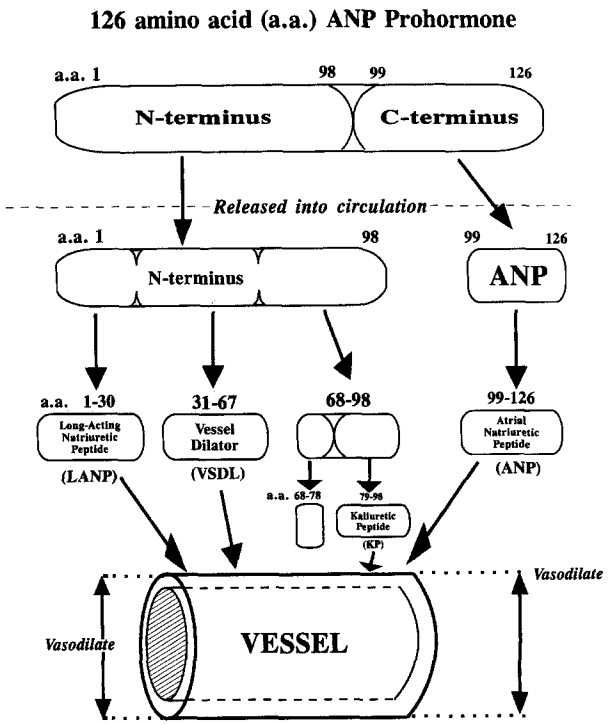

Fig 1. Origination of LANP, vessel dilator, kaliuretic peptide, and ANP from the ANP prohormone. LANP consisting of aa 1-30, vessel dilator aa 31-67, and kaliuretic peptide aa 79-98 originate from the amino terminus of the ANP prohormone, whereas ANP consisting of aa 99-126 originates from the carboxy terminus of this 126-aa prohormone. Each of these peptide hormones circulate as distinct entities, and all have diuretic properties and decrease blood pressure via vasodilation of blood vessels.[11,12]

tol phosphoglycan (IPG) and defective IPG-regulated intracellular glucose metabolism, which may contribute to the insulin resistance of diabetic GK rats.[36] Chronic activation of protein kinase C also appears to contribute to impaired glycogen synthesis and insulin resistance in GK rats.[37,38] In addition to insulin resistance, glucose-stimulated insulin secretion is impaired in the GK rat.[39] Accordingly, the GK model, like type 2 diabetes in humans, is characterized as having an impairment of both insulin action and secretion.

Ten-week-old male and female GK rats weighing 229 ± 7 g from this colony were used. Age- and weight-matched (235 ± 9 g) nondiabetic Wistar rats served as controls. All animals were fed pelleted standard Purina (St Louis, MO) rat chow ad libitum with free access to tap water. They were kept in temperature-controlled (24°C) rooms with alternating 12-hour periods of light and darkness. All rats were nonketotic.

This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (Publication No. 85-23, revised 1985). The investigation was approved by the Animal Care Committee of the University of South Florida and the James A. Haley Veterans Hospital, Tampa, FL.

Materials

Materials were reagent-grade or better. Serum glucose levels were measured after a tail snip by the glucose oxidase method (Boehringer Mannheim, Indianapolis, IN). At the time of experimentation, serum glucose levels were about 7.7 to 8.3 mmol/L in nondiabetic Wistar rats and 11.1 to 13.8 mmol/L in GK rats. Serum insulin levels measured with purified rat insulin as a standard (Novo, Copenhagen, Denmark) were approximately twofold higher in GK rats compared with Wistar rats.[36-38] All chemicals were purchased from Sigma Chemical (St Louis, MO) unless otherwise specified.

Measurement of LANP, Vessel Dilator, Kaliuretic Peptide, and ANP

Each of the blood samples were collected into chilled 5-mL EDTA tubes to prevent proteolytic breakdown of any peptides that might be present. These samples were transported on ice and immediately centrifuged at 3,000 × *g* for 15 minutes. After centrifugation, the plasma was transferred to 10 × 75-mm plastic tubes and stored in a −80°C ultralow freezer until assay. At the time of assay, plasma samples from Wistar and GK rats were thawed, and each plasma sample was extracted with 100% ethanol (1:2 dilution), vortexed, and allowed to stand at 4°C for 30 minutes.[11,40] The radioimmunoassays (RIAs) to measure LANP, vessel dilator, kaliuretic peptide, and ANP levels have been described previously in detail.[11,40] The intraassay coefficient of variation for LANP, vessel dilator, kaliuretic peptide, and ANP was 4.8%, 5.3%, 5.5%, and 5.7%, respectively. The interassay coefficient of variation was 8% for both LANP and vessel dilator and 7.3% and 7.6% for ANP and kaliuretic peptide. Recovery was examined by adding synthetic unlabeled LANP, vessel dilator, kaliuretic peptide, and ANP at 100, 200, and 400 pg/mL to pooled plasma. The recovery for LANP, vessel dilator, kaliuretic peptide, and ANP was 83% ± 13%, 100% ± 9%, 89% ± 12%, and 92% ± 11%, respectively. The lowest detectable concentrations of LANP, vessel dilator, kaliuretic peptide, and ANP were 40, 35, 5, and 1.4 fmol/tube, whereas nonspecific binding was 2.1%, 2.5%, 2.7%, and 2.9% in the respective RIAs. None of these peptide RIAs cross-react with each other. The human ANP antibodies have 100% cross-reactivity in both human and rat plasma. The vessel dilator antibody has 100% cross-reactivity in humans but only 14% cross-reactivity in the rat. The vessel dilator values in the Results are the actual measured values, but because of 14% cross-reactivity, these are approximately one seventh of the concentration of vessel dilator present in the circulation of rats. Serial dilution of pooled plasma has shown excellent parallelism of standards and unknowns in these assays.[30,40]

Blood Volume, Hematocrit, BUN, and Creatinine

Blood volume was measured in GK (218 ± 12 g) and Wistar (212 ± 3 g) rats at 10 weeks of age by infusing 100,000 cpm (1 μCi, 37 Bq) ^{125}I-albumin in phosphate buffer, pH 7.5, via a catheter in the internal jugular vein while each animal was anesthetized. Fifteen minutes later, blood samples were obtained via another port in the internal jugular vein catheter. These samples were analyzed in a gamma counter (TM Anolytic Gamma Trac 1193; Elk Grove Village, IL) for radioactivity. The known amount of ^{125}I-albumin infused was divided by the counts per minute obtained in the 15-minute postinfusion blood samples to determine the blood volume of GK and nondiabetic Wistar rats (n = 6 for each). BUN and serum creatinine levels were measured spectrophotometrically with kits from Sigma after deproteinizing the samples with 3% trichloroacetic acid. The hematocrit was calculated from the packed red blood cells in capillary tubes after centrifugation. Plasma volume was estimated from the known blood volume and hematocrit by the following formula: plasma volume = blood volume × ([100 − Hct]/100).

Echocardiography

At 10 weeks of age, all animals underwent transthoracic echocardiography. The echocardiograms were performed with a high-resolution, small-footprint neonatal transducer (7.0 mHz V7; Acuson, Mountain View, CA) with the anesthetized rat lying in the lateral decubitus position. Measurements were performed on two-dimensional triggered digitalized M-mode tracings from the short axis of the left ventricle at the level of the papillary muscle (resolution, 250 μm). All measurements were performed on three consecutive cardiac cycles. The left ventricular

diameter during systole (LVDs) and diastole (LVDd), percent fractional shortening, left ventricular ejection fraction (EF), posterior wall thickness at end diastole and systole (PWTd and PWTs), outer ventricular diameter during diastole (ODd), and interventricular septum thickness during diastole (IVSd) were measured according to the standards for M-mode measurements set by the American Society of Echocardiography as described previously from our laboratory.[41]

Statistics

All data are expressed as the mean ± SEM. Statistical analysis was performed using Student's *t* test. For statistical significance, a *P* value less than .05 was required.

RESULTS

Comparison of ANPs in GK Versus Wistar Rats

ANP, LANP, and vessel dilator were each threefold to sixfold higher in the circulation of GK rats compared with nondiabetic Wistar rats ($P < .01$). Kaliuretic peptide was twofold higher in the circulation of GK rats compared with Wistar rats ($P < .05$) (Fig 2). In each GK rat examined, the circulating concentration of ANP (range, 305 to 543 pg/mL) was significantly higher ($P < .01$) than the level in Wistar rats (range, 28 to 139 pg/mL). Likewise, vessel dilator (range, 343 to 614 pg/mL) was significantly ($P < .01$) higher in each GK rat compared with each Wistar rat (range, 97 to 177 pg/mL). There was no overlap

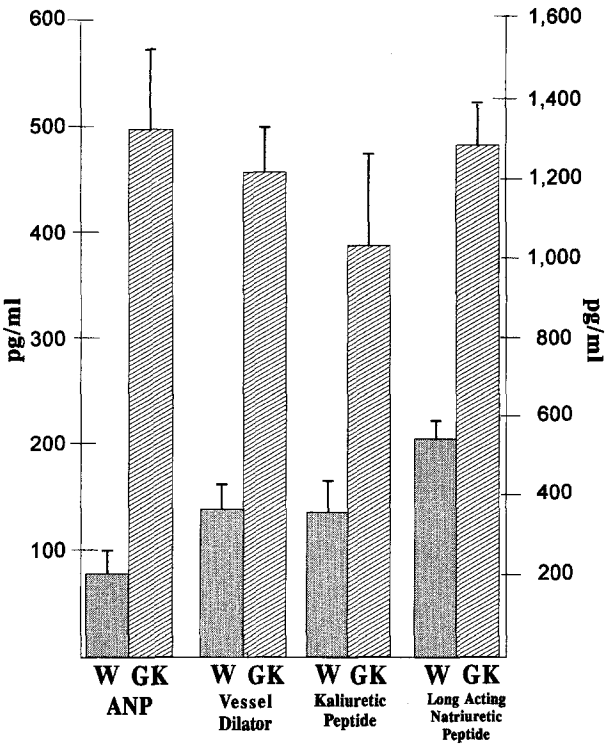

Fig 2. Comparison of the circulating concentration of ANPs in 10-week-old non-obese type 2 diabetic GK rats and 10-week-old healthy nondiabetic Wistar (W) rats. The increase of ANP, vessel dilator, and LANP in GK rats (n = 10) was significant at $P < .01$ and the significance for kaliuretic peptide was $P < .05$ as evaluated by Student's *t* test to determine which means of these peptides were different from the circulating concentrations in Wistar rats (n = 6). The scale for ANP, vessel dilator, and kaliuretic peptide is on the left ordinate, and the scale for LANP is on the right ordinate.

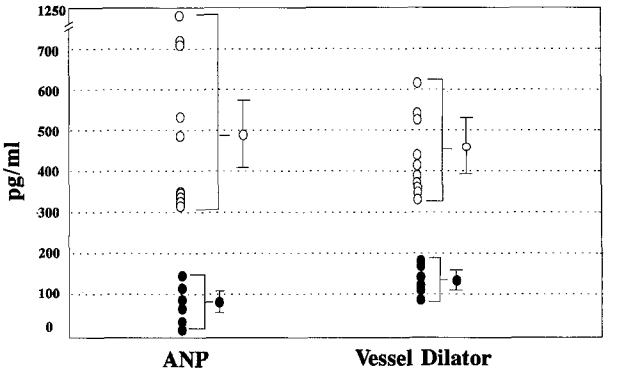

Fig 3. ANP and vessel dilator plasma concentrations are higher in each of 10 GK rats (○) compared with 6 Wistar rats (●). There was no overlap in the circulating concentration of these 2 peptides between GK and Wistar rats each examined at 10 weeks of age. The difference in the concentrations was significant at $P < .01$ between GK and Wistar rats as evaluated by Student's *t* test.

of the circulating concentrations of ANP or vessel dilator in comparing GK and Wistar rats (Fig 3).

The concentration of LANP was significantly ($P < .01$) higher in GK rats (range, 1,050 to 1,770 pg/mL) versus Wistar rats (range, 484 to 644 pg/mL). There was no overlap in the circulating concentration of LANP or kaliuretic peptide in comparing Wistar and GK rats. Kaliuretic peptide, although not as markedly different as the other three peptide hormones, was still significantly higher ($P < .05$) in GK rats (range, 219 to 1,475 pg/mL) compared with Wistar rats (range, 103 to 176 pg/mL). One of the GK rats had a much higher kaliuretic peptide concentration than the other GK rats. When this animal was removed from the analysis, kaliuretic peptide was 271 ± 36 pg/mL in GK rats and 134 ± 33 pg/mL in Wistar rats, a significant difference ($P < .05$) (Fig 4).

Renal Function

Serum creatinine levels were significantly ($P < .01$) elevated in GK rats compared with Wistar rats (Table 1). Thus, the serum creatinine of 12 GK rats was 1.77 ± 0.20 mg/dL, compared with 0.61 ± 0.03 mg/dL for Wistar rats (n = 6). To be sure that these creatinine levels in Wistar rats were not unduly low, we measured serum creatinine in Sprague-Dawley rats as another control (0.48 ± 0.01 mg/dL). With respect to the elevated serum creatinine in GK rats, the majority of the animals had a creatinine of 1.14 to 1.87 mg/dL (1.41 ± 0.3 mg/dL, n = 9), which reflects a 48% to 67% decrease in renal function in GK rats compared with Wistar and Sprague-Dawley rats. Three GK rats had a serum creatinine of 2.7 to 3.06 mg/dL (2.84 ± 0.14), which reflects a 79% decrease in renal function compared with Wistar rats. BUN levels in GK rats were similarly increased twofold to fivefold ($P < .01$) compared with Wistar rats. BUN correlated with creatinine in that nine GK rats with a mean serum creatinine of 1.41 ± 0.3 mg/dL had a mean BUN of 23 ± 1 mg/dL, whereas three GK rats with a serum creatinine of 2.84 ± 0.14 had a mean BUN of 32 ± 1 mg/dL. As a group, 12 GK rats had BUN levels threefold to fourfold higher than Wistar and Sprague-Dawley rats (Table 1).

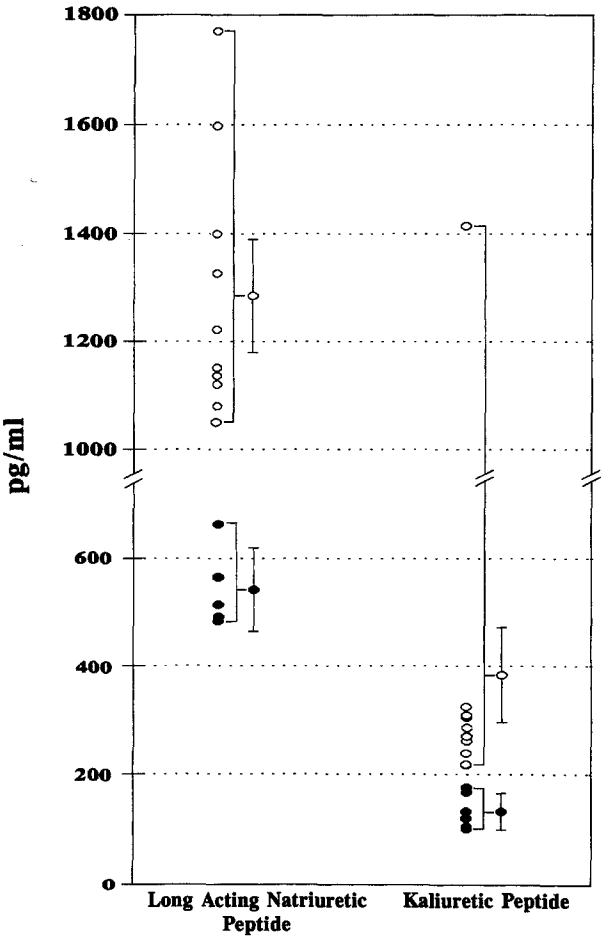

Fig 4. LANP and kaliuretic peptide are higher in each individual GK rat (○) compared with each Wistar rat (●). The difference in the concentrations between GK (n = 10) and Wistar (n = 6) rats each examined at 10 weeks of age when $P < .01$ for LANP and $P < .05$ for kaliuretic peptide was evaluated by Student's *t* test.

Blood Volume

Blood volume was found to be 1.7-fold higher ($P < .01$) in GK rats (16.2 ± 0.4 mL, 81 ± 2 mL/kg) compared with Wistar rats (9.5 ± 0.3 mL, 45 ± 2 mL/kg). The plasma volume of GK rats was 10.85 ± 0.23 mL, whereas the plasma volume of Wistar rats was 5.33 ± 0.16 mL ($P < .01$) (Fig 5).

Hematocrit

The hematocrit of Wistar rats was 44% ± 2%, whereas the hematocrit of GK rats was 33% ± 1%.

Echocardiographic Evaluation of Ventricular Dilation in GK Versus Wistar Rats

Echocardiographic measurement of the ventricles of GK rats and age- and weight-matched Wistar rats was performed to determine if the increased blood and plasma volume in GK rats compared with Wistar rats result in dilation of the ventricle of GK rats as a possible cause of the increase in ANPs in the circulation of GK rats. Left ventricular diameter during systole (LVDs) and posterior wall thickness during diastole (PWTd) were slightly but not significantly increased in GK versus Wistar rats (Table 2 and Fig 6). Likewise, there was no significant difference between the interventricular septum thickness in diastole (IVSd), left ventricular diameter during systole (LVDs), posterior wall thickness during systole (PWTs), or left ventricular ejection fraction (EF) between GK and Wistar rats. The outer left ventricular diameter during diastole (ODd) and left ventricular diameter during diastole (LVDd) were actually decreased ($P < .05$) in GK rats compared with Wistar rats. Fractional shortening (percent) was decreased slightly in GK rats (48% ± 3%) compared with Wistar rats (53% ± 3%), consistent with depressed left ventricular systolic function, but this did not reach a level of statistical significance. GK rats did not have a significantly increased heart rate compared with Wistar rats. The relative left ventricular wall thickness was greater in GK compared with Wistar rats. We also noted on visual inspection in the basal state when placing the intravenous catheters that the internal jugular veins were markedly distended in GK rats compared with Wistar rats (Table 2 and Fig 6).

DISCUSSION

ANPs: LANP, vessel dilator, kaliuretic peptide, and ANP were markedly elevated in the non-obese type 2 diabetic rat model (GK rat) compared with age- and weight-matched nondiabetic Wistar rats from which the GK colony was derived. The finding of increased ANP in the circulation of this

Table 1. Evaluation of Renal Function of GK Rats Versus Wistar and Sprague-Dawley Rats

Rat Group	Creatinine (mg/dL)	BUN (mg/dL)
Wistar		
1	0.47	3
2	0.61	8
3	0.71	11
4	0.67	9
5	0.56	5
6	0.61	8
Mean ± SEM	0.61 ± 0.03	7.3 ± 1.2
GK		
1	1.56	24
2	1.20	22
3	1.14	21
4	1.87	26
5	1.44	23
6	1.67	25
7	1.27	22
8	1.38	24
9	1.13	19
10	2.76	31
11	2.73	32
12	3.06	33
Mean ± SEM	1.77 ± 0.20*	25.1 ± 1.3*
Sprague-Dawley		
1	0.47	4
2	0.51	7
3	0.46	4
4	0.49	8
5	0.50	7
6	0.47	6
Mean ± SEM	0.48 ± .01	6.0 ± 0.7

*Significantly different *v* Wistar and Sprague-Dawley.

spontaneous model of diabetes suggests that STZ per se in the STZ-induced diabetic model is not the cause of elevated ANP found previously in this model.[1-6] Rather, from the present investigation, it would appear that increased ANP in the circulation is an integral part of the diabetic state. Accordingly, the increase in ANP in the GK rat may cause, at least in part, the hyperfiltration characteristically observed in the early phases of diabetes mellitus as demonstrated previously by others.[1,4,6]

LANP, vessel dilator, and kaliuretic peptide, which have not been previously investigated in any spontaneous or drug-induced model of diabetes, were also found to be markedly elevated in non-obese type 2 diabetic GK rats. Since these peptide hormones are synthesized in the same ANP gene[7,41] and are released simultaneously with ANP in response to a change in plasma volume[20,30,31] and atrial pressure,[31] one would expect their plasma levels to increase along with ANP[7-9] unless there is altered processing of the ANP prohormone in diabetes. The increase in all four of these peptide hormones is evidence against an altered processing of the ANP prohormone in type 2 diabetes.[7-9] The amount of increase for each of these ANPs in GK rats is remarkable in that the circulating concentration of each of the peptides is very high, with ANP (sixfold) being as high as the level observed in class III New York Heart Association congestive heart failure of both rats and humans.[40-43]

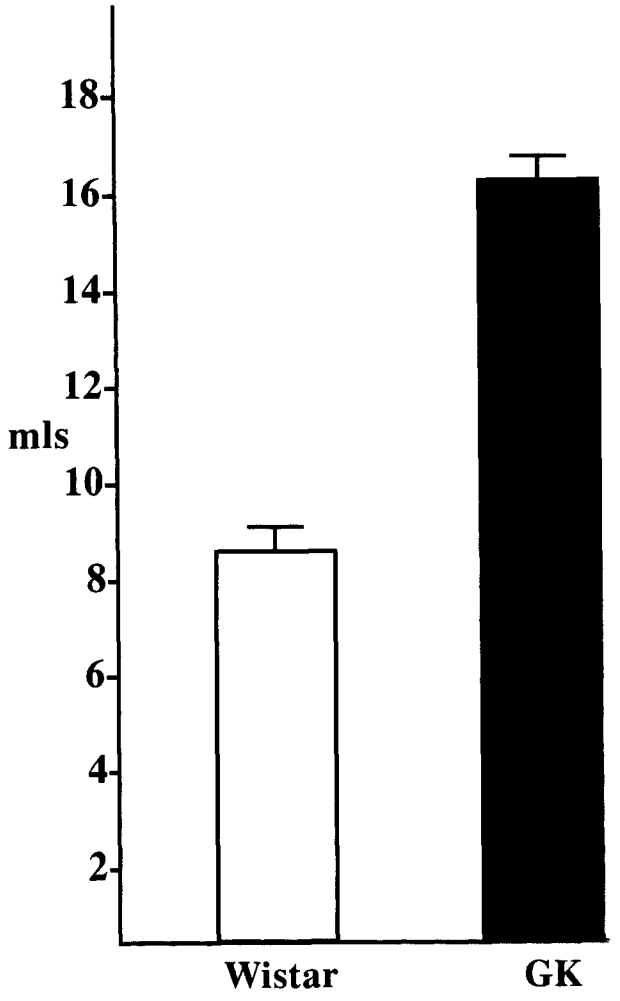

Fig 5. Blood volume is 1.7-fold higher in GK rats compared with Wistar rats. This increase in blood volume in 10-week-old diabetic GK rats compared with 10-week-old Wistar rats was significant at $P < .01$ as evaluated by Student's *t* test (n = 6 for each).

Table 2. Echocardiographic Parameters of Hemodynamic Function in Diabetic GK and Nondiabetic Wistar Rats

Parameter	Wistar	GK
Heart rate (beats/min)	350 ± 14	336 ± 22
PWTd (mm)	1.5 ± 0.1	1.6 ± 0.1
PWTs (mm)	2.0 ± 0.1	1.9 ± 0.2
IVSd (mm)	1.6 ± 0.1	1.4 ± 0.1
ODd (mm)	9.3 ± 0.1	8.9 ± 0.4
LVDd (mm)	6.2 ± 0.3	6.1 ± 0.4
LVDs (mm)	2.9 ± 0.2	3.1 ± 0.4
FS%	53 ± 3	48 ± 3
EF%	56 ± 2	57 ± 3
RWT	0.49 ± 0.03	0.55 ± 0.05

NOTE. Data represent the mean ± SEM for 5 individual measurements in each group. There was not any significant difference in hemodynamic function when each variable was compared in GK rats age- and weight-matched Wistar rats by Student's *t* test.

Abbreviations: PWTd, posterior wall thickness during diastole; PWTs, posterior wall thickness during systole; IVSd, interventricular septum thickness in diastole; ODd, outer ventricular diameter during diastole; LVDd, left ventricular diameter during diastole; LVDs, left ventricular diameter during systole; EF%, left ventricular ejection fraction as a percent; RWT, relative wall thickness; FS, fractional shortening (obtained from the following formula: ([LVDd − LVDs]/LVDs) × 100.

The respective ANPs were found to be increased relatively early chronologically in the GK rat and may therefore contribute to the hyperfiltration found in the early phase of the diabetic state. Since the concentrations of LANP, vessel dilator, kaliuretic peptide, and ANP were all increased, each may contribute to the hyperfiltration found early in diabetics. However, the percent of hyperfiltration in diabetics due to each of the respective ANPs is presently uncertain. It is known that blocking ANP effects with specific ANP antiserum has resulted in only a partial correction of the hyperfiltration of diabetic rats, suggesting that other factors in addition to ANP contribute to hyperfiltration in diabetes.[4] LANP, vessel dilator, and kaliuretic peptide have potent vasorelaxant and diuretic properties similar to ANP, but their biologic effects last longer, ie, 6 hours, compared with 30 minutes or less for ANP.[11,43] Previous studies have suggested that the vasodilatory prostaglandin E_2 is important in mediating the hyperfiltration of diabetes.[44,45] Prostaglandin E_2 synthesis in the kidney is increased by LANP, vessel dilator, and kaliuretic peptide, but not by ANP.[26-29] Indomethacin, a nonspecific prostaglandin blocker, inhibits prostaglandin E_2 synthesis and corrects 50% of the renal hyperfiltration of diabetes.[4] Of further note, the combined infusion of ANP antiserum and indomethacin completely corrects the hyperfiltration of diabetes.[4] Thus, if one blocks the effects of LANP, vessel dilator, and kaliuretic peptide with indomethacin[26,27] and the effects of ANP with ANP antiserum,[4] one should be able to fully correct the hyperfiltration of diabetes. This would suggest that these four peptide hormones are collectively the primary

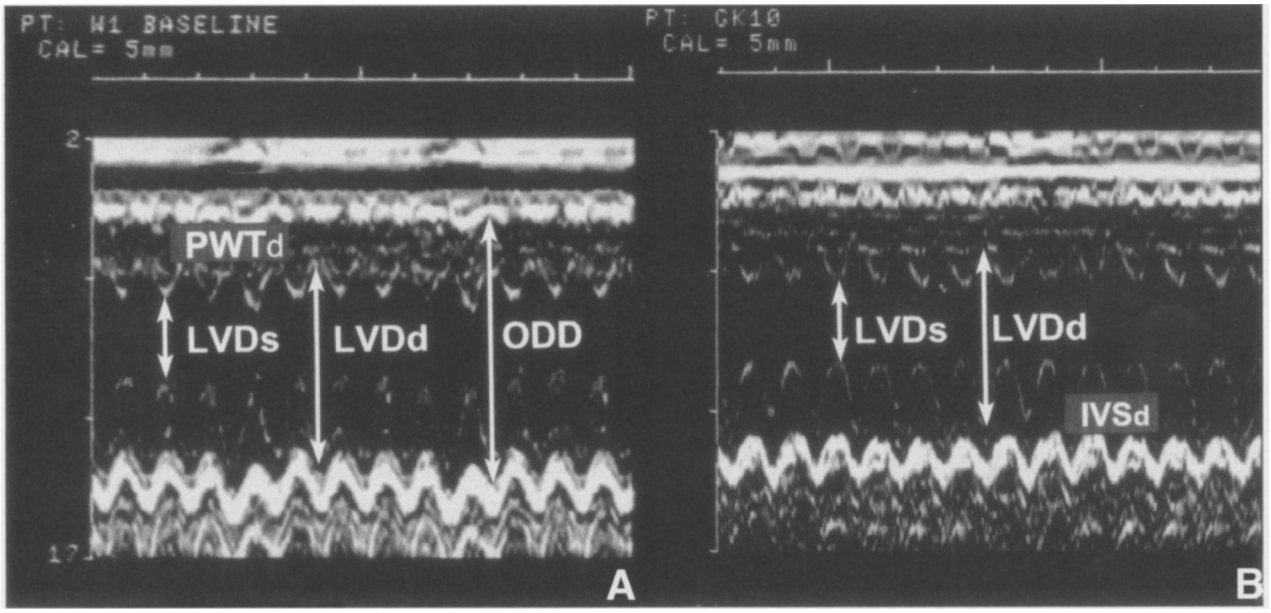

Fig 6. Ultrasound of ventricles of nondiabetic Wistar rat (A) compared with age-matched diabetic GK rat (B). IVSd, interventricular septum during diastole; PWTd, posterior wall thickness during diastole. The outer diameter (ODD) and left ventricular diameter during diastole (LVDd) and systole (LVDs) were not significantly increased in 10-week-old GK rats versus 10-week-old Wistar rats as evaluated by Student's *t* test.

mediators of the hyperfiltration that occurs in early diabetes mellitus.

The present investigation demonstrates that the GK rat develops a major complication of diabetes, ie, renal failure, at a surprisingly early age. Although the GK rat has been used for over 20 years (since 1975) as a model of type 2 diabetes mellitus,[33] renal function of the GK rat has not been evaluated previously. Renal function of the GK rat was found to be decreased 48% to 79% compared with Wistar and Sprague-Dawley rats. This marked decrease was found in all GK animals by 10 weeks of age. Further evaluation of younger GK rats showed that decreased renal function to the extent found with the 10-week-old rats was present in all GK rats at 6 weeks of age. Evaluation of older GK rats (at 36 weeks) showed that renal function was decreased similarly to that of 10-week GK rats but had not deteriorated further.

Humans with renal failure have markedly elevated circulating concentrations of ANPs.[14] The decreased renal function of GK rats is thus likely to serve as a major contributor to the increases in circulating ANPs found in this animal model of diabetes. When the kidney does not metabolize these peptides and excrete them into the urine, they accumulate in the circulation in high concentrations.[7-9,14] It is also important to note that the concentrations of atrial peptides in renal failure are affected very little by hemodialysis but vary with the plasma volume of the individual with renal failure.[46]

In addition to renal failure resulting in decreased metabolic processing of ANPs, the presently observed increase in the blood and plasma volumes of GK rats is probably important in mediating the increase in circulating concentrations of the respective ANPs. Increased plasma volume with atrial stretch is generally the main cause of the simultaneous release of ANPs in healthy animals[31] and humans.[20,30] Plasma volume is also thought to be an important contributor to the increase of ANP in humans with diabetes.[47] In an alloxan-induced rabbit model of diabetes, it has been found that plasma ANP and glucose concentrations are directly correlated ($P < .001$), with higher glucose levels associated with higher ANP levels.[48] Since the increase in plasma glucose in diabetes may directly contribute to the increase in plasma volume, glucose itself may contribute to the increase in ANPs in diabetics.

In diabetic rats, the main site of increased ANP gene expression is reported to be the heart ventricle as opposed to the atria,[49] similar to what is found in animals with congestive heart failure.[41] For this reason, we examined whether ventricle stretch might be the cause of the increased ANPs found in the circulation of the diabetic animals. There was no evidence of dilation of the ventricle of GK rats compared with nondiabetic Wistar rats during systole or diastole, which is distinctly different from congestive heart failure, wherein dilated ventricles contribute to increases in ANPs.[41] The echocardiographic data of the present investigation suggest that ventricle stretch in diabetic animals was not a major contributing factor to the increased ANPs found in diabetic rats. Rather, increased blood and plasma volumes, which stretch the atria of the heart, appear to be a main mediator of the increase in the release of ANPs in this spontaneous model of type 2 diabetes.

In summary, LANP, vessel dilator, kaliuretic peptide, and ANP were significantly elevated in the circulation of non-obese type 2 diabetic (GK) rats compared with healthy nondiabetic animals. A likely cause for the increase in ANPs within the circulation was identified, ie, increased blood and plasma volumes resulting in stretching of the atria of the heart. Further, renal failure was found to occur surprisingly early in this model of type 2 diabetes mellitus and probably contributed importantly to the increase in plasma volume. Prospective studies of

earlier time points will be important in this spontaneous model of diabetes mellitus to determine when renal failure begins in this model and to clarify the relationship of glucose elevation with changes in circulating ANP concentrations, renal failure, and volume expansion.

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