

# Converting Enzyme Inhibition and the Glomerular Hemodynamic Response to Glycine in Diabetic Rats

LARRY A. SLOMOWITZ,\* ORJAN W. PETERSON,<sup>†</sup> and SCOTT C. THOMSON<sup>†</sup>

\*Baylor School of Medicine, Houston, Texas; and <sup>†</sup>Department of Medicine, Division of Nephrology/Hypertension, Veterans Affairs Medical Center and University of California, San Diego, California.

**Abstract.** GFR normally increases during glycine infusion. This response is absent in humans and rats with established diabetes mellitus. In diabetic patients, angiotensin-converting enzyme inhibition (ACEI) restores the effect of glycine on GFR. To ascertain the glomerular hemodynamic basis for this effect of ACEI, micropuncture studies were performed in male Wistar-Froemter rats after 5 to 6 wk of insulin-treated streptozotocin diabetes. The determinants of single-nephron GFR (SNGFR) were assessed in each rat before and during glycine infusion. Studies were performed in diabetics, diabetics after 5 d of ACEI (enalapril in the drinking water), and weight-matched controls. Diabetic rats manifest renal hypertrophy and glomerular hyperfiltration but not glomerular capillary hypertension. ACEI reduced glomerular capillary pressure, increased

glomerular ultrafiltration coefficient, and did not mitigate hyperfiltration. In controls, glycine increased SNGFR by 30% due to increased nephron plasma flow. In diabetics, glycine had no effect on any determinant of SNGFR. In ACEI-treated diabetics, the SNGFR response to glycine was indistinguishable from nondiabetics, but the effect of glycine was mediated by greater ultrafiltration pressure rather than by greater plasma flow. These findings demonstrate that: (1) The absent response to glycine in established diabetes does not indicate that renal functional reserve is exhausted by hyperfiltration; and (2) ACEI restores the GFR response to glycine in established diabetes, but this response is mediated by increased ultrafiltration pressure rather than by increased nephron plasma flow.

Diabetes mellitus is the leading cause of end-stage renal disease in the United States. Glomerular hyperfiltration occurs early in the course of diabetes and has been implicated in the pathogenesis of diabetic nephropathy (1). The humoral basis underlying diabetic glomerular hyperfiltration remains incompletely understood despite known abnormalities in several paracrine systems (reviewed in reference 1).

Inhibitors of angiotensin-converting enzyme (ACEI) and restriction of dietary protein both have salutary effects on the progression of renal disease in diabetes mellitus (1,2). The benefits of a low protein diet and ACEI to the kidney are putatively linked to the mitigation of glomerular hyperfiltration. However, in a previous study performed in rats 1 wk after the onset of streptozotocin diabetes, glycine infusion (a surrogate for protein feeding) did not cause single-nephron GFR (SNGFR) to increase, whereas in diabetic rats treated with ACEI, SNGFR did increase in response to glycine (3). Similarly, prior treatment with ACEI has been found to magnify the renal vasodilatory response to amino acid infusion in diabetic patients with normal renal function, but not in nondiabetic humans (4). In light of the presumed hemodynamic basis for

the salutary effects of protein restriction and ACEI on the diabetic kidney, the finding that ACEI allows SNGFR to increase in response to glycine presents a paradox.

One potential solution to this paradox might lie in the timing of the prior animal studies relative to the course of diabetes. During the first 2 wk of streptozotocin diabetes, plasma renin activity (PRA) is elevated above normal, whereas beyond 4 wk of streptozotocin diabetes, both PRA (5) and the total kidney content of angiotensin II (6) are suppressed below normal. Therefore, it is possible that the effect of ACEI on the response to glycine is different in rats with early diabetes compared to rats with established diabetes. For this reason, we performed micropuncture experiments to examine the effect of ACEI on the glomerular hemodynamic response to glycine infusion in rats 5 to 6 wk after induction of streptozotocin diabetes.

## Materials and Methods

All animal experimentation described herein was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals. Adult male Wistar-Froemter rats were made diabetic by streptozotocin (STZ; 65 mg/kg intraperitoneally; Sigma, St. Louis, MO) dissolved in sodium citrate buffer, pH 4.2. Two days later, the glucose concentration was determined in tail blood samples, and only those animals with blood glucose levels >300 mg/dl were included in further experiments. Diabetic rats were treated daily with protamine zinc insulin (0.5 to 1.5 IU subcutaneously in late afternoon; Anpro Pharmaceutical, Arcadia, CA) to adjust blood glucose levels at approximately 350 mg/dl. The animals were allowed free access to a regular rat pellet diet (sodium 0.44%, chloride 0.63%, potassium 0.97%, protein 21%) and tap water. Six weeks after onset of diabetes mellitus, rats were divided into two groups, one receiving 15 mg/L

Received June 19, 1998. Accepted January 17, 1999.

Correspondence to Dr. Scott C. Thomson, Associate Professor of Medicine, Division of Nephrology/Hypertension, University of California, San Diego, and San Diego Veterans Affairs Medical Center, 3350 La Jolla Village Drive, San Diego, CA 92161-9151. Phone: 619-552-8585, ext. 3793; Fax: 619-552-7549; E-mail: sthompson@ucsd.edu

1046-6673/1007-1447

Journal of the American Society of Nephrology

Copyright © 1999 by the American Society of Nephrology

enalapril in the drinking water and the other receiving tap water alone. Five days later, nonfasted rats were prepared for micropuncture. Nondiabetic rats fed the same diet served as controls.

### Micropuncture Protocol

Micropuncture was performed under Inactin (100 mg/kg intraperitoneally; Research Biochemicals, Natick, MA) anesthesia after tracheostomy (PE 240); catheterization (PE 50) of the right jugular vein, left femoral artery, urinary bladder, and left ureter; and surgical preparation of the left kidney for micropuncture according to protocols described previously in publications from this laboratory (7). Body temperature was regulated by a Servo-controlled heating table. All studies were conducted under euvolemic conditions, with animals receiving donor rat plasma (11 ml/kg body wt over 60 min followed by continuous infusion at 2 ml/kg per h) as replacement for surgical losses. An additional infusion of Ringer's saline containing 80  $\mu$ Ci/ml [ $^3$ H]-inulin as a marker of glomerular filtration was infused continuously at 1.5 ml/h. After completion of the preparatory surgery, animals were allowed 60 min to equilibrate before beginning micropuncture. After the first period of micropuncture, an infusion of L-glycine (2.66 M in Ringer's saline) was started at a rate of 1.4 ml/h. After 20 min for reequilibration, the second period of micropuncture was begun. This protocol was established based on past experience with glycine infusion and was intended to maintain isovolemia throughout the second experimental period (8). Adequacy of ACEI was confirmed by absence of a hypertensive response to 100 ng of angiotensin I given as an intravenous bolus at the end of the second micropuncture period.

Arterial BP was monitored from the femoral artery catheter via a P23Db Gould Statham pressure transducer and Statham chart recorder. A Servo-nulling pressure device using micropipettes filled with hypertonic saline was used to measure hydrostatic pressures in glomerular capillaries ( $P_{GC}$ ), Bowman's space ( $P_{BS}$ ), efferent arterioles ( $P_E$ ), and proximal tubules both before ( $P_{FF}$ ) and after ( $P_{SF}$ ) placement of an obstructing downstream oil block. Systemic blood was sampled from the femoral artery. Efferent arteriolar blood was obtained by direct micropuncture. A microadaptation of the Lowry technique (9) was used to determine the protein concentrations of systemic ( $C_A$ ) and efferent ( $C_E$ ) arteriolar plasma. Plasma oncotic pressure ( $\pi$ ) was calculated from protein concentration by the Landis-Pappenheimer equation (10). Nephron filtration fraction was computed from  $C_A$  and  $C_E$ . Inulin clearance and volumetric measurement of fluid collected from late proximal tubules were used to calculate SNGFR and late proximal flow rate ( $V_{LP}$ ). Absolute (APR) and fractional (FPR) rates of proximal reabsorption were calculated from SNGFR and  $V_{LP}$ . Each experimental period involved five separate determinations of  $P_{FF}$ ,  $P_{SF}$ ,  $P_E$ , SNGFR,  $V_{LP}$ , APR, and FPR. During each experimental period, blood was collected from three separate efferent arterioles and twice from the femoral artery.  $P_{GC}$  and  $P_{BS}$  were measured by direct glomerular puncture according to the accessibility of surface glomeruli. Glomeruli of diabetic rats were observed frequently to blanch when punctured, often requiring 1 to 2 min for the restoration of blood flow. For this reason, and to economize on the time allowed to elapse during an experimental period, full sets of data for  $P_{GC}$  were not obtained by direct puncture in every experiment. Values for  $P_{GC}$  and  $\Delta P$ , as calculated from the period mean  $P_{SF}$ ,  $P_{FF}$ , and  $C_A$ , were thus used in subsequent calculations and statistical comparisons.

### Mathematical Models

The determinants of SNGFR are as follows: nephron plasma flow,  $SNPF = (SNGFR \times C_E / (C_E - C_A))$ ; afferent effective filtration

pressure,  $EFP_A = P_{SF} - P_{FF}$ ; and glomerular ultrafiltration coefficient,  $LpA$ , such that;

$$LpA = \frac{SNGFR}{\int_0^1 EFP(x) \times dx}$$

where  $x$  is the axial position along a nondimensionalized glomerular capillary,

$$EFP(x) = P_{SF} - P_{FF} + \pi_A - \pi(x)$$

$$\pi(x) = 1.73C(x) + 0.28C^2(x)$$

and the plasma protein concentration,  $C(x)$ , is calculated according to standard formulas (11) with the boundary conditions  $C(0) = C_A$  and  $C(1) = C_E$ .

Preglomerular and efferent arteriolar vascular resistance were calculated as:

$$R_A = \frac{(BP - P_{SF} - \pi_A) \times (1 - Hct)}{SNPF}$$

$$R_E = \frac{(P_{SF} + \pi_A - P_E)}{\left(\frac{SNPF}{(1 - Hct)} - SNGFR\right)}$$

### Statistical Analyses

The main goal of these studies was to test for the effect of diabetes and diabetes + enalapril on the response to glycine. To take best advantage of the paired nature of the experiments, the effects of treatments were analyzed by one-way ANOVA with design for repeated measures (12). For parameters measured more than once during an experimental period (SNGFR,  $V_{LP}$ , FPR, APR,  $EFP_A$ ,  $P_E$ ), the mean for that period was used. For these parameters, groups were also compared by standard two-way ANOVA using individual measurements. The results of analyses by these two methods were similar. Individual intergroup comparisons were by  $t$  test, paired or unpaired as appropriate.

Because  $C_A$  was noted to decline during the second micropuncture period, a separate analysis was performed to determine the degree to which the apparent effects of glycine on the other determinants of SNGFR were influenced by changes in  $C_A$ . This was accomplished through a stepwise multivariate regression analysis to define the degree of interdependence among the variables affecting glomerular filtration. Multivariate regression formulas were calculated with proprietary software (Systat<sup>®</sup>, SPSS, Inc.). Separate analyses were performed for  $EFP_A$  and  $\Delta P$  to correlate changes in these parameters with simultaneous changes in  $\pi_A$ ,  $LpA$ ,  $SNPF$ , diabetes, enalapril, and glycine. The threshold for elimination of individual terms during stepwise regression was set at  $P > 0.15$ .

To confirm that the specific relationships among the individual determinants of SNGFR detected by this multivariate regression analysis are not unique to the present data, similar analyses were performed on micropuncture data published by Brenner (13) and Blantz (14), who previously manipulated systemic oncotic pressure to characterize the influence of oncotic pressure changes on the other determinants of SNGFR. For this analysis, multivariate regression formulas were generated from the combined data of Brenner (13) and Blantz (14) ( $n = 78$  micropuncture experiments). Separate analyses were performed for  $EFP_A$  and  $\Delta P$  to correlate changes in these parameters

with combined changes in  $\pi_A$ , LpA, SNPF, the method of changing  $C_A$ , and laboratory where the experiments were performed (Brenner *versus* Blantz). The threshold for elimination of individual terms during the stepwise regressions was set at  $P > 0.15$ .

**Results**

Data were gathered from control rats ( $n = 6$ ), diabetic rats ( $n = 6$ ), and diabetic rats treated with enalapril ( $n = 7$ ). Control, diabetic, and ACEI-diabetic rats weighed  $300 \pm 12$ ,  $258 \pm 8$ , and  $296 \pm 9$  g, respectively. Kidneys from diabetic rats weighed substantially more than kidneys from control rats, and kidney weight was not diminished by 5 d of enalapril ( $1.06 \pm 0.04$ ,  $1.40 \pm 0.06$ , and  $1.54 \pm 0.08$  g for control, diabetic, and ACEI-diabetic rats, respectively). Normalizing kidney weight to body weight strengthened the impression of hypertrophy in diabetes, which was not affected by 5 d of ACEI ( $3.5 \pm 0.1$ ,  $5.4 \pm 0.2$ , and  $5.2 \pm 0.2$  g/kg, respectively). Mean arterial pressure at the time of micropuncture was not different between the groups (Table 1). To assess the adequacy of ACEI in enalapril-treated rats, the systemic arterial pressure response to a 100-ng bolus of angiotensin I was tested in four ACEI-diabetic rats and four control rats. In control rats, angiotensin I caused an immediate transient increment of  $38 \pm 2$  mmHg in systemic arterial BP, whereas angiotensin I administered to enalapril-treated diabetic rats had an effect on arterial pressure that was not distinguishable from saline placebo in any animal. Diabetic rats were moderately hyperglycemic, and blood glucose concentration was not significantly affected by enalapril ( $324 \pm 54$  *versus*  $283 \pm 56$  mg/dl,  $P = \text{NS}$ ). Micropuncture data are presented in Table 1 and discussed below.

*Baseline Glomerular Hemodynamics*

Diabetes increased basal SNGFR by approximately 20% ( $P < 0.003$  *versus* control). Basal hyperfiltration was not significantly affected by 5 d of enalapril. The hyperfiltration associated with diabetes could not be ascribed to any single determinant of SNGFR and was the product of minor increases in effective filtration pressure and ultrafiltration coefficient. When normalized to body weight, nephron plasma flow and SNGFR in diabetic rats exceeded controls by 15 and 38%, respectively. Diabetes was associated with a group mean increase in glomerular capillary pressure of only 2.3 mmHg ( $P = \text{NS}$ , diabetes *versus* control). In other words, diabetes did not cause significant glomerular capillary hypertension in this model. However, treatment of diabetic rats with enalapril reduced glomerular capillary pressure by 7 mmHg ( $P < 0.04$  diabetes + enalapril *versus* diabetes) without lowering systemic arterial BP.

As filtration occurs along the glomerular capillary, the capillary plasma oncotic pressure increases. Under circumstances in which the capillary oncotic pressure increases enough to approach the transcapillary hydrostatic pressure gradient, the capillary comes into a state of "filtration pressure equilibrium." When glomerular ultrafiltration coefficient is calculated for nephrons in filtration pressure equilibrium, the value derived is a minimum estimate of the true ultrafiltration coefficient. In the current experiments, two of the seven enalapril-treated animals

Table 1. Micropuncture data<sup>a</sup>

| Group and Period                                                                                                  | MAP     | $P_{GC}$            | $\Delta P$ | $EFP_A$             | SNGFR               | APR      | SNPF                  | FF                | $R_A$                    | $R_E$           | LpA                        |
|-------------------------------------------------------------------------------------------------------------------|---------|---------------------|------------|---------------------|---------------------|----------|-----------------------|-------------------|--------------------------|-----------------|----------------------------|
|                                                                                                                   |         | (mmHg)              |            |                     |                     | (nl/min) | (%)                   | (mmHg/nl per min) | (mmHg/nl per min)        | (ml/s per mmHg) |                            |
| Control                                                                                                           |         |                     |            |                     |                     |          |                       |                   |                          |                 |                            |
| baseline                                                                                                          | 118 ± 1 | 54 ± 2              | 38 ± 1     | 19 ± 1              | 41 ± 2 <sup>b</sup> | 13 ± 1   | 171 ± 24              | 26 ± 2            | 0.21 ± 0.02              | 0.13 ± 0.03     | 0.058 ± 0.008              |
| +glycine                                                                                                          | 118 ± 3 | 57 ± 3              | 36 ± 2     | 18 ± 1              | 54 ± 3 <sup>c</sup> | 12 ± 2   | 217 ± 26 <sup>c</sup> | 26 ± 3            | 0.16 ± 0.03 <sup>c</sup> | 0.12 ± 0.02     | 0.068 ± 0.008              |
| Diabetes                                                                                                          |         |                     |            |                     |                     |          |                       |                   |                          |                 |                            |
| baseline                                                                                                          | 111 ± 6 | 57 ± 2              | 40 ± 2     | 21 ± 2              | 49 ± 3              | 16 ± 2   | 172 ± 19              | 30 ± 3            | 0.17 ± 0.01              | 0.14 ± 0.02     | 0.067 ± 0.009              |
| +glycine                                                                                                          | 110 ± 6 | 60 ± 5              | 40 ± 3     | 22 ± 2              | 51 ± 5              | 16 ± 4   | 160 ± 16              | 32 ± 2            | 0.17 ± 0.02              | 0.16 ± 0.02     | 0.064 ± 0.012              |
| Diabetes + ACEI                                                                                                   |         |                     |            |                     |                     |          |                       |                   |                          |                 |                            |
| baseline                                                                                                          | 119 ± 5 | 50 ± 2 <sup>b</sup> | 38 ± 2     | 19 ± 1              | 52 ± 5              | 19 ± 3   | 167 ± 27              | 33 ± 3            | 0.24 ± 0.04              | 0.13 ± 0.02     | 0.106 ± 0.023 <sup>b</sup> |
| +glycine                                                                                                          | 121 ± 3 | 55 ± 3              | 39 ± 2     | 24 ± 1 <sup>c</sup> | 68 ± 4 <sup>c</sup> | 19 ± 2   | 171 ± 15              | 40 ± 3            | 0.21 ± 0.02              | 0.13 ± 0.01     | 0.101 ± 0.022              |
| <i>P</i> values associated from the cross-term in two-way ANOVA reflecting differences in the response to glycine |         |                     |            |                     |                     |          |                       |                   |                          |                 |                            |
| control <i>versus</i> diabetes                                                                                    | NS      | NS                  | NS         | NS                  | 0.006               | NS       | 0.002                 |                   | 0.05                     | 0.02            | NS                         |
| diabetes <i>versus</i> diabetes + ACEI                                                                            | NS      | NS                  | NS         | 0.07                | 0.007               | NS       | NS                    |                   | NS                       | NS              | NS                         |

<sup>a</sup> MAP, mean arterial pressure;  $P_{GC}$ , glomerular capillary pressure;  $\Delta P$ , transcapillary pressure gradient;  $EFP_A$ , effective afferent filtration pressure; SNGFR, single-nephron GFR; APR, absolute proximal reabsorption; SNPF, single-nephron plasma flow; FF, filtration fraction;  $R_A$ , afferent arteriolar resistance;  $R_E$ , efferent arteriolar resistance; LpA, glomerular ultrafiltration coefficient; NS, not significant; ACEI, angiotensin-converting enzyme inhibition.

<sup>b</sup>  $P < 0.05$  *versus* baseline diabetes.

<sup>c</sup>  $P < 0.05$  *versus* baseline, same group.

manifested filtration pressure equilibrium both before and during glycine infusion. One control animal was in filtration pressure equilibrium during glycine infusion. All other animals, including all of the diabetic rats, were in filtration pressure disequilibrium throughout both periods of micropuncture. Glomerular ultrafiltration coefficient did not differ between controls and untreated diabetic rats. Glomerular ultrafiltration coefficient was increased by treatment with enalapril ( $P < 0.03$ ). Furthermore, since only the enalapril group contained animals in filtration pressure equilibrium, the estimated impact of enalapril on the group mean ultrafiltration coefficient represents a lower limit of the true effect of enalapril.

During glycine infusion, there was a tendency for absolute proximal reabsorption to be greater in diabetes, and to be further enhanced by treatment with enalapril. However, these effects did not achieve statistical significance, nor did differences in fractional reabsorption.

### Glomerular Hemodynamic Response to Glycine

In control animals, glycine infusion caused SNGFR to increase by  $31 \pm 4\%$  ( $P < 0.001$ ). This increase was mediated by an increase in nephron plasma flow ( $30 \pm 10\%$ ,  $P < 0.03$ ). There was a minor tendency for ultrafiltration coefficient to increase during glycine, but this effect was not statistically significant. The afferent effective filtration pressure and nephron filtration fraction were also unaffected by glycine in control animals. These effects of glycine on the individual determinants of SNGFR in euvoletic control animals are the same as those observed in several prior published studies performed in this laboratory (reviewed in reference (15)).

In animals with established diabetes and glomerular hyperfiltration, glycine had no effect on SNGFR or any of its determinants. The responses of diabetic and control animals to glycine were compared by ANOVA with design for repeated measures. By this test, statistically significant differences between control and diabetic animals were detected for the effects of glycine on SNGFR, nephron plasma flow, nephron blood flow, afferent arteriolar resistance, efferent arteriolar resistance, and late proximal flow. The  $P$  values associated with these various effects of diabetes on the response to glycine are recorded along with the group data in Table 1.

Treatment of hyperfiltering diabetic animals with enalapril restored the effect of glycine on SNGFR, such that SNGFR increased by  $34 \pm 10\%$  ( $P = 0.01$ ). The effect of glycine on SNGFR in enalapril-treated diabetic rats was significantly greater than the effect of glycine on SNGFR in nontreated diabetic rats ( $P = 0.017$ ) and not different from the effect of glycine on SNGFR in controls. However, whereas the effect of glycine in control animals was mediated by an increase in nephron plasma flow, the increment in SNGFR during glycine infusion in enalapril-treated diabetics was mediated by an increase in afferent ultrafiltration pressure ( $30 \pm 15\%$ ,  $P = 0.05$  by paired  $t$  test) during glycine infusion. Nephron plasma flow and glomerular ultrafiltration coefficient were not affected by glycine in enalapril-treated diabetics. By ANOVA, the effect of glycine on afferent effective filtration pressure was significantly different in enalapril-treated diabetics *versus*

controls ( $P = 0.05$ ), confirming a statistically significant difference between the mechanisms whereby glycine caused SNGFR to increase in control rats *versus* enalapril-treated diabetic rats.

### Role of Oncotic Pressure in the Response to Glycine

In most animals, the systemic plasma protein concentration decreased during the second period. The mean  $\pm$  SEM differences in  $C_A$  between first and second periods were  $0.2 \pm 0.2$ ,  $0.4 \pm 0.2$ , and  $0.6 \pm 0.2$  g/dl for control, diabetic, and diabetic + enalapril rats, respectively. These intergroup differences were not statistically significant ( $P = 0.25$  by ANOVA for the effect of group on the change in  $C_A$  between periods considering all groups, and  $P = 0.34$  for the effect of enalapril on the difference between periods in protein concentration among diabetics). Nonetheless, changes in  $C_A$  were a potential confounding influence on the determinants of SNGFR. To assess the magnitude and significance of this confounding influence, stepwise multivariate regression analyses for the present data and for previously published data were performed as described in Materials and Methods. Among the data obtained from Brenner (13) and Blantz (14), there was a very strong positive correlation between  $\Delta P$  and  $\pi_A$ , a strong negative correlation between  $\Delta P$  and  $Lp_A$ , and no correlation between  $\Delta P$  and SNPF. There was a weak negative correlation between  $EFP_A$  and  $\pi_A$ , a strong negative correlation between  $EFP_A$  and  $Lp_A$ , and no correlation between  $EFP_A$  and SNPF. The correlation coefficients were not appreciably altered by including terms for the laboratory in which the experiments were performed or the protocol the investigators used to manipulate  $C_A$ . The specific regression coefficients and associated  $P$  values are shown in Table 2.

The regression coefficients and associated  $P$  values for multivariate analysis of the present data are shown in Table 3. Analysis of the present data yielded results similar to those described above for previously published data. In other words, there was a strong positive correlation between  $\Delta P$  and  $\pi_A$ , a strong negative correlation between  $\Delta P$  and  $Lp_A$ , and no correlation between  $\Delta P$  and SNPF. There was no correlation between  $EFP_A$  and  $\pi_A$ , a strong negative correlation between  $EFP_A$  and  $Lp_A$ , and no correlation between  $EFP_A$  and SNPF. The individual regression coefficients from the equations that were fit to the present data were remarkably similar to the regression coefficients from the equations that were fit to the earlier data of Brenner (13) and Blantz (14). Therefore, the sensitivity of  $\Delta P$  and  $Lp_A$  to changes in plasma oncotic pressure, which was described for normal rats by Brenner (13) and Blantz (14), appears to be unaltered by diabetes.

### Glomerular Capillary Pressure by Stop-Flow and Direct Measurement

Data were generated in the course of these experiments to permit a comparison between the direct and stop-flow methods for determining  $P_{GC}$ . In 16 periods of micropuncture,  $P_{GC}$  was measured by both direct capillary puncture of one or more glomeruli (usually one) and by stop-flow measurements in three or more (usually five) nephrons. Within each period, the



Table 2. Multivariate regression applied to published micropuncture data from Brenner (13) and Blantz (14)<sup>a</sup>

| Original model tested: $\Delta P = \pi_A + LpA + SNPF + Protocol + Lab$ |                                              |                     |               |
|-------------------------------------------------------------------------|----------------------------------------------|---------------------|---------------|
| Dependent Variable                                                      | Independent Terms in Multivariate Regression |                     |               |
|                                                                         | $\pi_A$ (mmHg)                               | LpA (nl/s per mmHg) | SNPF (nl/min) |
| $\Delta P$ (mmHg)                                                       |                                              |                     |               |
| Regression coefficient                                                  | $0.84 \pm 0.09$                              | $-72 \pm 10$        | $\sim 0$      |
| <i>P</i> value associated with regression coefficient                   | $2 \times 10^{-10}$                          | $1 \times 10^{-10}$ | 0.750         |
| Original model tested: $EFP_A = \pi_A + LpA + SNPF + Protocol + Lab$    |                                              |                     |               |
| Dependent Variable                                                      | Independent Terms in Multivariate Regression |                     |               |
|                                                                         | $\pi_A$ (mmHg)                               | LpA (nl/s per mmHg) | SNPF (nl/min) |
| $EFP_A$ (mmHg)                                                          |                                              |                     |               |
| Regression coefficient                                                  | $-0.16 \pm 0.09$                             | $-72 \pm 10$        | $\sim 0$      |
| <i>P</i> value associated with regression coefficient                   | 0.10                                         | $2 \times 10^{-11}$ | 0.750         |

<sup>a</sup> “Protocol” is a categorical variable that refers to the experimental design for manipulating plasma protein concentration. “Lab” is a categorical variable that refers to the laboratory where experiments were performed (Brenner *versus* Blantz). The regression coefficients for  $\pi_A$ , LpA, and SNPF did not change significantly when these categorical variables were removed from the analysis. The results shown here were obtained after removing these categorical variables. Abbreviations as in Table 1.

direct and stop-flow measurements were from different nephrons. Linear regression of the mean value for  $P_{GC}$  as calculated from stop-flow data *versus* the mean value for  $P_{GC}$  as measured by direct capillary puncture for each individual period yielded a *y*-intercept of  $-0.15$  mmHg, a slope of 1.02, and a correlation coefficient,  $r^2 = 0.66$ ,  $P < 2 \times 10^{-6}$ . These data are depicted in Figure 1.

The mean  $\pm$  SEM residual (vertical distance between computed  $P_{GC}$  and the regression line in Figure 1) was  $3.84 \pm 0.66$  mmHg. The inter-nephron SD among stop-flow pressures within an experimental period was  $3.0 \pm 0.3$  mmHg for 33 periods in which three to five stop-flow measurements were made. Therefore, most of the differences between measured and calculated  $P_{GC}$  can be accounted for by normal inter-nephron heterogeneity. The *y*-intercept of the regression line passed within 0.15 mmHg of the origin. This suggests that plasma oncotic pressures determined from the micro-Lowry protein data and Landis–Pappenheimer equation were quite accurate.

## Discussion

The increase in glomerular filtration that normally occurs during glycine infusion is absent in hyperfiltering established diabetes. Treatment with enalapril does not mitigate the underlying hyperfiltration but does restore the capacity for glomerular filtration to increase during glycine infusion. This suggests that the blunted response to glycine in established diabetes is not due to exhaustion of the renal functional reserve and suggests that there is some derangement of the renin-angiotensin system in established diabetes that prevents the normal modulation of GFR. However, while enalapril normalizes the increment in SNGFR during glycine infusion, the glomerular

hemodynamic pattern of this response differs from the pattern in nondiabetics. The present data in nondiabetic control animals are consistent with the published literature according to which the effect of glycine on glomerular filtration can be completely accounted for by an increase in nephron plasma flow (15). On the contrary, in enalapril-treated rats with established diabetes, glycine does not affect nephron plasma flow, but causes SNGFR to increase by increasing ultrafiltration pressure. Inasmuch as glycine infusion is a model for protein feeding and inasmuch as increased transcapillary pressure is thought to be deleterious to the diabetic kidney, the fact that enalapril allows ultrafiltration pressure to increase during glycine infusion suggests that a salutary effect of ACEI might be undermined by protein feeding.

According to the standard model for glomerular filtration, there are four parameters that determine SNGFR. These include  $\Delta P$ , SNPF, LpA, and  $\pi_A$ . The fact that SNGFR can be calculated from these parameters is a mathematical truism that requires no assumptions regarding interactions among the parameters that may occur in physiology. However, failing to account for effects that the individual determinants of SNGFR exert on each other can lead to incorrect conclusions regarding the cause of a change in SNGFR. For example, if the four determinants of SNGFR are treated as independent variables for the purpose of interpreting the present data, then SNGFR would appear to increase during glycine in ACEI-treated diabetics purely as the result of a decrease in  $\pi_A$ . However, both clinicians and micropuncturists are intuitively aware that causing  $\pi_A$  to decline is not an effective means for increasing GFR. The reason for this is that the determinants of SNGFR are not independent of each another and, therefore, the fact that  $EFP_A$  can be computed from  $\Delta P$  and  $\pi_A$  should not be taken to

Table 3. Multivariate regression applied to data from the present studies<sup>a</sup>

| Original model tested: $\Delta P = \pi_A + \text{LpA} + \text{SNPF} + \text{Diabetes} + \text{Enalapril} + \text{Glycine}$ |                                              |                     |               |
|----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|---------------------|---------------|
| Dependent Variable                                                                                                         | Independent Terms in Multivariate Regression |                     |               |
|                                                                                                                            | $\pi_A$ (mmHg)                               | LpA (nl/s per mmHg) | SNPF (nl/min) |
| $\Delta P$ (mmHg)                                                                                                          |                                              |                     |               |
| Regression coefficient                                                                                                     | $0.91 \pm 0.17$                              | $-47 \pm 11$        | $\sim 0$      |
| P value associated with regression coefficient                                                                             | $7 \times 10^{-6}$                           | 0.0001              | 0.705         |
| Original model tested: $EFP_A = \pi_A + \text{LpA} + \text{SNPF} + \text{Diabetes} + \text{Enalapril} + \text{Glycine}$    |                                              |                     |               |
| Dependent Variable                                                                                                         | Independent Terms in Multivariate Regression |                     |               |
|                                                                                                                            | $\pi_A$ (mmHg)                               | LpA (nl/s per mmHg) | SNPF (nl/min) |
| $EFP_A$ (mmHg)                                                                                                             |                                              |                     |               |
| Regression coefficient                                                                                                     | $\sim 0$                                     | $-47 \pm 11$        | $\sim 0$      |
| P value associated with regression coefficient                                                                             | 0.612                                        | 0.0001              | 0.573         |

<sup>a</sup> The regression coefficients for  $\pi_A$ , LpA, and SNPF were not significantly affected when diabetes, enalapril, and glycine were removed from the analysis. The results shown were obtained after removing those categorical variables. Abbreviations as in Table 1.

suggest that changes in  $EFP_A$  can be predicted from changes in  $\pi_A$ .

In the past, both Brenner (13) and Blantz (14) studied the effects of changing plasma oncotic pressure on  $\Delta P$ , SNPF, and

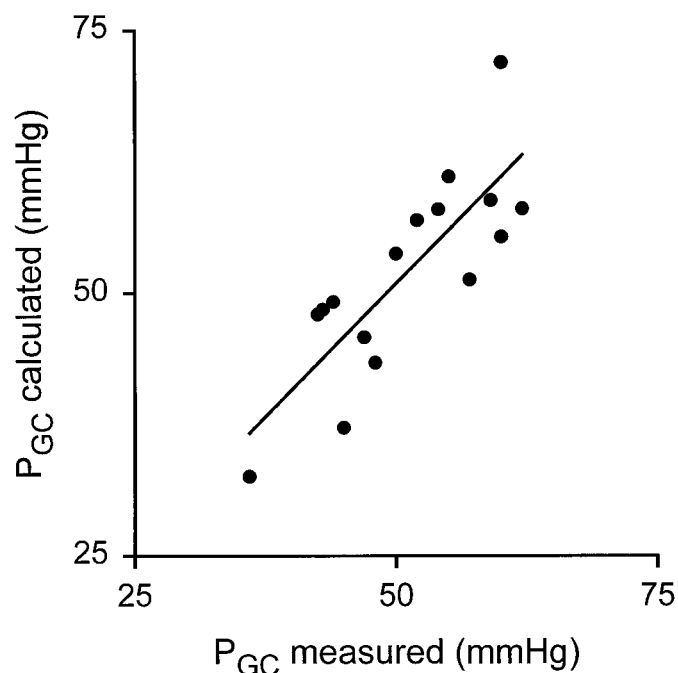


Figure 1. Glomerular capillary pressure calculated from tubular stop-flow pressure and plasma protein concentration (y-axis) versus glomerular capillary pressure as determined by direct capillary micropuncture (x-axis). Each point represents the mean value for an experimental period. Data are pooled from all groups. Stop-flow and direct micropuncture measurements are from different nephrons. The regression equation:  $y = -0.15 + 1.02x$ .

LpA. These investigators drew upon a wide variety of infusion and exchange protocols to induce changes in systemic plasma protein concentration. Both of these investigators discovered that the four determinants of SNGFR are not independent of one another. Specifically, they discovered that, regardless of the experimental means for invoking a change in  $\pi_A$ , a change in  $\pi_A$  causes a parallel change in  $\Delta P$ , and reciprocal change in ultrafiltration coefficient (LpA). In contrast, neither SNPF nor  $EFP_A$  are directly affected by changes in  $\pi_A$ . When analogous multivariate regression analyses were performed to provide direct comparison of the present data to the previous works of Brenner and Blantz, *vis-a-vis* interactions among the determinants of SNGFR, the results were virtually identical (Tables 2 and 3). Furthermore, the regression coefficients linking  $\Delta P$  to  $\pi_A$  are close enough to unity to render  $EFP_A$  independent of  $\pi_A$ . Therefore, glycine could not have caused  $EFP_A$  to increase in enalapril-treated diabetics without affecting the glomerular microvasculature. Using the regression coefficient for  $\pi_A$  to correct for confounding effects of inconstant  $\pi_A$  in each individual animal suggests that had  $\pi_A$  remained constant between first and second periods in each rat, glycine would have caused  $\Delta P$  to change by  $-0.9 \pm 1.4$ ,  $0.8 \pm 1.1$ , and  $4.6 \pm 2.1$  mmHg, in control, diabetic, and ACEI-diabetic animals, respectively.

Inhibition of ACE was confirmed in these studies by the absence of a BP response to angiotensin I and by the clearcut effects of ACEI on the kidney. The finding that ACEI did not reduce BP in these normotensive diabetic rats suggests that the systemic renin-angiotensin system was not a main determinant of BP in these rats with established diabetes that were prepared for micropuncture according to the standard euvoletic protocol. This is not surprising since plasma renin activity is normally suppressed at this stage of experimental diabetes (5). Incidentally, BP was also unaffected by captopril in a group of

normotensive diabetic patients in whom captopril restored the responsiveness of GFR to amino acid infusion (4).

The renin-angiotensin system is compartmentalized and angiotensin II serves a number of paracrine functions in the kidney. The particular pool of angiotensin II that is most relevant to the present study must act locally within the glomerular microvasculature. Indeed, in diabetic rats with reduced whole kidney ACE activity, there is enhanced immunostaining for ACE in the glomeruli and renal vasculature (16). Therefore, the prior observation that the whole kidney angiotensin II content is significantly reduced in established diabetes (6) is not necessarily at odds with the increase in LpA, the reduction in  $P_{GC}$ , or the potentiation of a glomerular hemodynamic response to glycine in diabetic rats receiving enalapril. In fact, these findings suggest a major tonic influence of endogenous angiotensin II within the glomeruli of these rats, even though these rats did not manifest glomerular capillary hypertension. An increase in the tonic influence of angiotensin II over the glomerular ultrafiltration coefficient is characteristic of several other models of nephron hyperfiltration that are prone to develop glomerulosclerosis (reviewed in reference 17).

Most angiotensin II in the kidney exists outside of the glomerulus, and the present experiments may suggest an overall reduction in kidney angiotensin II content since enalapril did not appear to affect the proximal tubular response to glycine in established diabetes. The effect of glycine on proximal tubular reabsorption has been examined in several rat models. A common thread among these is that glycine seems to selectively reduce proximal reabsorption whenever the angiotensin II:nitric oxide ratio is high (reviewed in reference (15)). In early diabetes, for example, glycine exerted an inhibitory effect on proximal reabsorption that was reversed with angiotensin II blockade (3). In contrast, the present experiments in established diabetes revealed no such effects of glycine on the proximal tubule with or without enalapril. Proximal reabsorption is normally stimulated by sub-nanomolar concentrations of angiotensin II and inhibited by supra-nanomolar concentrations of angiotensin II (18). Glycine appears to unmask the latter condition, which prevails in early diabetes, but does not prevail in established diabetes.

The current study was designed to examine the effects of diabetes on the response to glycine infusion rather than to examine the effects of diabetes on basal glomerular function. Body weight averaged somewhat less in the untreated diabetic group, but because the response to glycine differed qualitatively between the groups, the main results are presented without normalizing for body weight. Nonetheless, diabetic glomerular hyperfiltration is demonstrated unequivocally. On the other hand, the failure to observe any difference in absolute nephron plasma flow between controls and the smaller diabetics does not necessarily suggest that diabetes had no effect on nephron plasma flow. However, the present data do suggest that glomerular hyperfiltration was not purely a consequence of greater nephron plasma flow. In the majority of published studies addressing this issue, glomerular hyperfiltration is accompanied by increased renal plasma flow. Overall, however, the literature conveys a more consistent correlation between

GFR and filtration fraction than between GFR and plasma flow, and there are published examples in both human (19,20) and experimental (21–24) diabetes in which diabetic hyperfiltration occurs in the absence of an increase in renal plasma flow. Analogous inconsistencies exist in the literature regarding the role of glomerular capillary hypertension in diabetic hyperfiltration (reviewed in reference (25)). Such discrepancies would be resolved if it could be shown that hyperfiltration, *per se*, is required to minimize an error signal in some physiologic feedback loop. Then it would not be surprising that hyperfiltration is a constant finding in diabetes, whereas the route to hyperfiltration varies according to other superimposed physiologic circumstances.

In summary, glycine infusion causes nephron GFR to increase in normal rats but not in hyperfiltering rats with established streptozotocin diabetes. This does not suggest that renal functional reserve is exhausted by hyperfiltration in animals with established streptozotocin diabetes since converting enzyme inhibition increases the glomerular ultrafiltration coefficient and normalizes the increment in nephron GFR during glycine infusion. The response to glycine in ACE-inhibited established diabetes is mediated by greater effective filtration pressure, whereas the responses in normal rats or ACE-inhibited rats with early diabetes are mediated by greater nephron plasma flow. These findings suggest that there are subtle differences between early and established diabetes that only become apparent when studies are performed at the level of the single nephron and that converting enzyme inhibition might not truly restore the glomerular microvasculature to a state of hemodynamic normalcy.

## Acknowledgments

This work was supported by the Roderick Duncan McDonald Fund and the Department of Veterans Affairs. Expert technical assistance was provided by Margarida Mendonca and Ser Khang.

## References

1. Bryan GT, Hostetter TH: The renal hemodynamic basis of diabetic nephropathy. *Semin Nephrol* 17: 93–100, 1997
2. Breyer JA: Therapeutic interventions for nephropathy in type I diabetes mellitus. *Semin Nephrol* 17: 114–123, 1997
3. DeNicola L, Blantz RC, Gabbai FB: Renal functional reserve in the early stage of experimental diabetes. *Diabetes* 41: 267–273, 1992
4. Slomowitz LA, Hirschberg R, Kopple JD: Captopril augments the renal response to an amino acid infusion in diabetic adults. *Am J Physiol* 255: F755–F762, 1988
5. Kikkawa R, Kitamura E, Fujiwara Y, Haneda M, Shigeta Y: Biphasic alteration of renin-angiotensin-aldosterone system in streptozotocin-diabetic rats. *Renal Physiol* 9: 187–192, 1986
6. Vallon V, Wead LM, Blantz RC: Renal hemodynamics and plasma and kidney angiotensin II in established diabetes mellitus in rats: Effect of sodium and salt restriction. *J Am Soc Nephrol* 5: 1761–1767, 1995
7. Blantz RC, Tucker BJ: Measurements of glomerular dynamics. In: *Methods in Pharmacology*, edited by Martinez-Maldonado M, New York, Plenum, 1978, pp 141–163

8. DeNicola L, Thomson SC, Wead LM, Brown MR, Gabbai FB: Arginine feeding normalizes the renal response to glycine in cyclosporine treated rats. *J Clin Invest* 92: 1859–1865, 1993
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265–275, 1951
10. Landis EM, Pappenheimer JR: Exchange of substances through the capillary walls. In: *Handbook of Physiology*, edited by Orloff J, Berliner RW, Washington DC, American Physiological Society, 1964, pp 961–1034
11. Thomson SC, Tucker BJ, Gabbai FB, Blantz RC: Functional effects on glomerular hemodynamics of short-term cyclosporine in male rats. *J Clin Invest* 83: 960–969, 1989
12. Wilkinson L: *Systat: The System for Statistics*, Evanston, IL, Systat, Inc., 1989
13. Baylis C, Ichikawa I, Willis WT, Wilson CB, Brenner BM: Dynamics of glomerular ultrafiltration. IX. Effects of plasma protein concentration. *Am J Physiol* 232: F58–F71, 1977
14. Tucker BJ, Blantz RC: Effects of glomerular filtration dynamics on the glomerular permeability coefficient. *Am J Physiol* 240: F245–F254, 1981
15. Gabbai FB, DeNicola L, Garcia GE, Blantz RC: Role of angiotensin in the regulation of renal response to proteins. *Semin Nephrol* 15: 396–404, 1995
16. Anderson S, Jung FF, Ingelfinger JR: Renal renin-angiotensin system in diabetes: Functional, immunohistochemical, and molecular biological correlations. *Am J Physiol* 265: F477–F486, 1993
17. Slomowitz L: Tubuloglomerular feedback in chronic renal failure. *Nephron* 45: 264–271, 1987
18. Harris PJ, Young JA: Dose dependent stimulation and inhibition of proximal tubular sodium reabsorption by angiotensin II in the rat kidney. *Pflügers Arch* 366: 295–297, 1977
19. Ditzel J, Junker K: Abnormal glomerular filtration rate, renal plasma flow, and protein excretion in recent and short-term diabetics *Br Med J* 2: 13–19, 1972
20. Stalder G, Schmid R: Severe functional disorders of glomerular capillaries and renal hemodynamics in treated diabetes mellitus during childhood. *Ann Paediatr* 193: 129–138, 1959
21. Vallon V, Kirschenmann D, Wead LM, Lortie MJ, Satriano J, Blantz RC, Thomson SC: Effect of chronic salt loading on kidney function in early and established diabetes mellitus in rats. *J Lab Clin Med* 130: 76–82, 1997
22. Tucker BJ, Anderson CM, Thies S, Collins RC, Blantz RC: Glomerular hemodynamic alterations during acute hyperinsulinemia in normal and diabetic rats. *Kidney Int* 42: 1160–1168, 1992
23. Tucker BJ, Collins RC, Ziegler MG, Blantz RC: Disassociation between glomerular hyperfiltration and extracellular volume in diabetic rats. *Kidney Int* 39: 1176–1183, 1991
24. Komers R, Cooper ME: Acute renal hemodynamic effects of ACE inhibition in diabetic hyperfiltration: Role of kinins. *Am J Physiol* 268: F588–F594, 1995
25. O'Donnell MP, Kasiske BL, Keane WF: Glomerular hemodynamic and structural alterations in experimental diabetes mellitus. *FASEB J* 8: 2339–2347, 1988