Vascular Reactivity to Angiotensin II and to Norepinephrine in Diabetic Subjects

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SUMMARY

Vascular responsiveness to infused angiotensin II and to norepinephrine was determined in 14 normal subjects and two groups of diabetic subjects, 16 with no clinically detectable diabetic complications and 14 with diabetic retinopathy but no clinical evidence of nephropathy. All were maintained on a 100-mEq.-Na-100-mEq.-K diet. Serum electrolytes, 24-hour urinary sodium, creatinine clearance, and plasma renin activity did not differ significantly among the groups. Group mean baseline diastolic pressure in those with retinopathy was higher than in normal subjects but not significantly different from that of uncomplicated diabetics. The pressor dose of angiotensin II (ng./kg./min. to increase diastolic blood pressure 20 mm. Hg) for each group respectively was 11.5 ± 0.9, 12.9 ± 1.3, and 8.3 ± 1.3, and the slope of the dose-response curve (mm.Hg rise in blood pressure resulting from the infusion of 1 ng./kg./min. following the initial increment in blood pressure) was 2.0 ± 0.2, 1.6 ± 0.2, 3.3 ± 0.6. For norepinephrine, the pressor doses were 163 ± 24, 212 ± 21, and 123 ± 11 and slopes were 0.17 ± 0.03, 0.13 ± 0.02, and 0.20 ± 0.02. Neither diabetic group differed significantly from normal subjects. Diabetics with retinopathy were more sensitive to angiotensin II, pressor dose (P <0.059) and slope (P <0.02), and to norepinephrine, pressor dose (P <0.006) and slope (P =0.05) than those without complications. These data suggest that vascular reactivity is enhanced in diabetics with retinopathy. DIABETES *25*:268-74, April, 1976.

Disease affecting the macro- and microvasculature results in the major complications seen in patients with diabetes mellitus. Despite this, little is known concerning the reactivity of the diabetic vasculature to vasopressor substances. Altered vascular reactivity has been observed in other disease states associated with accelerated vascular disease. For example, patients with essential hypertension and with hypertension accompanied by chronic renal disease demonstrate an increased pressor sensitivity to angiotensin II, whereas those with renovascular or malignant hypertension are resistant to this peptide.[1-3] There is evidence suggesting that these altered responses may be related to circulating levels of endogenous renin and angiotensin II[1,4,5] and also to nonspecific changes in vascular reactivity as evidenced by parallel changes in norepinephrine and metaraminol sensitivity.[6] Any possible relationship between these changes in vascular reactivity and vascular pathology remains to be determined.

Alterations in vasopressor systems have been observed in diabetes mellitus. Plasma renin activity is decreased in diabetics with nephropathy[7] or ortho-

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static hypotension[8] and increased in diabetic ketoacidosis.[9] Circulating catecholamines may be decreased in diabetics with peripheral neuropathy.[10] Rats with alloxan-induced diabetes have decreased plasma renin activity accompanied by increased vascular responsiveness to angiotensin II but have normal responsiveness to norepinephrine.[11]

The present study is designed to determine vascular reactivity to angiotensin II and norepinephrine in diabetic patients with no clinically evident renal disease and to compare their responses with that of normal control subjects. The results suggest that diabetics with retinopathy have increased vascular responsiveness to both vasopressors over that of diabetics with no complications of their disease.

SUBJECTS

Written informed consent in accordance with the guidelines established by the National Institutes of Health was obtained from each of the 50 subjects studied. Each received a physical examination, had complete fundus photographs taken, and had normal studies done as follows: complete blood count, urinalysis, screening profile, chest x-ray, and electrocardiogram.

Normal subjects (controls) were studied as outpatients. Each had, in addition to the routine studies, a standard oral glucose tolerance test following three days of a high-carbohydrate diet. Fourteen had normal tests by the criteria of Kahn et al.,[12] and two were normal by the more liberal criteria of Wilkerson et al.[13]

Diabetic subjects hospitalized in the Diabetes Treatment Unit of the New England Deaconess Hospital were approached to participate in the study only if they had no evidence of coronary or other major vascular disease, moderate or severe hypertension, hepatic disease, or renal disease. Insulin was the only medication taken during the protocol. Diabetic subjects were divided into two groups, those without and those with retinopathy. The presence or absence of retinopathy (nonproliferative or proliferative) was determined from full-fundus photographs by two ophthalmologists.

Individual clinical data are presented in table 1. Group mean clinical and laboratory characteristics are presented in tables 2 and 3. With the exception of the higher diastolic blood pressure than that of controls and longer duration of diabetes in the group with retinopathy, no significant differences among the groups were found. Clinical evidence of peripheral neuropathy (altered deep-tendon reflexes and vibration or position sensation) was found in only four patients, two in group 2 and two in group 3. No patients had symptoms of autonomic neuropathy.

DETAILS OF PROTOCOL

All subjects ingested a 100-mEq.-Na-100-mEq.-K diet for at least two days before infusion studies. Normal subjects were instructed in this diet as outpatients. For diabetic subjects, the diet was prepared by the Dietary Department of the New England Deaconess Hospital. On the study day, plasma for renin activity determined by radioimmunoassay[8] was obtained prior to arising in the morning and again at noon after the patients had been upright for four hours (only upright plasma renin activity was assayed in control subjects). Twenty-four-hour urine was collected on the same day for determination of sodium, potassium, and creatinine. In the afternoon, with the subjects sitting, full-fundus photographs were obtained and an infusion of 5 per cent glucose and water was started with a constant-infusion pump (Harvard pump). Once the blood pressure, measured with a sphygmomanometer, was stabilized for a minimum of 10 minutes, the infusion was changed first to either norepinephrine (Levophed, 5 μg./ml.) or angiotensin II (Hypertensin, Ciba Pharmaceutical Co., Summit, N.J.) (300 ng./ml.), the pressor being alternated with each patient studied. Initial infusion rate was 0.3 ml./min. It soon became evident that diabetics with retinopathy had increased vasopressor sensitivity. Thereafter, more dilute solutions of norepinephrine (3 μg./ml.) and angiotensin II (150 ng./ml.) were used for patients with retinopathy observed by ophthalmoscopy. The rate of infusion was progressively increased every three to 10 minutes, with the blood pressure determined each minute. Each blood pressure was recorded along with the dosage of the respective pressor agent. Once the diastolic pressure had increased to approximately 20 mm. Hg for 10 minutes, infusions were stopped. Five per cent glucose and water was again infused until the blood pressure had stabilized at baseline levels for a minimum of 10 minutes, following which the alternate pressor agent was infused using the same procedure.

ANALYSIS OF DATA

Six patients were not included in the final analysis of the data but are included in table 1 (patients

TABLE 1

Individual clinical data, plasma renin activity (PRA), slope of dose-response curves, and pressor dose of angiotensin II (A-II) and norepinephrine (NE)

Patient	Age	Sex	Blood pressure (mm. Hg)	Duration of diabetes (years)	Creatinine clearance (ml./min.)	Urine Na (mEq./day)	PRA (ng./ml.)		Slope (dose/mm. BP rise)		Pressor dose (ng./kg./min)	
							Supine	Upright	A-II	NE	A-II	NE
Controls												
1	23	F	97/62		89	23		4.0	1.24	—	15.4	—
2	22	F	98/61		116	32		2.7	1.86	0.20	12.8	131
3	22	F	86/66		185	87		4.5	3.64	0.28	6.9	80
4	27	F	94/55		122	70		2.9	1.69	0.11	11.7	244
5	25	F	112/67		91	34		2.2	2.04	—	10.6	—
6	29	M	108/72		139	99		5.1	1.38	0.08	13.6	301
7	24	F	104/69		129	192		2.6	2.34	0.20	9.1	158
8	32	F	100/66		119	116		2.6	1.85	0.14	13.0	169
9	23	M	100/63		118	83		4.0	3.50	0.31	6.8	77
10	32	F	106/65		124	96		4.0	2.66	—	9.5	—
11	29	M	112/68		143	104		4.3	1.91	0.15	9.3	137
12	24	F	100/62		93	68		4.0	1.70	—	11.2	—
13	29	M	122/77		64	131		3.4	1.55	0.11	11.6	166
14	23	M	114/79		154	157		1.2	0.96	—	19.7	—
Diabetics—no retinopathy												
15	24	M	110/60	<1	136	59	4.2	9.6	1.03	0.09	13.9	285
16	42	F	103/75	<1	108	123	1.4	2.0	0.90	0.07	16.8	282
17	22	M	110/64	<1	138	53	0.3	1.8	1.93	0.15	9.9	172
18	32	F	118/78	<1	81	30	0.5	7.4	0.97	0.22	19.1	108
19	24	M	117/80	<1	112	33	2.0	4.6	1.78	0.19	10.0	124
20	23	M	107/75	<1	128	104	1.7	2.6	2.39	0.25	7.6	95
21	31	M	104/76	2	100	61	6.4	11.5	0.79	0.06	24.7	298
22	21	F	94/51	2	105	29	1.1	2.2	1.54	0.09	12.3	211
23	39	M	114/79	13	126	124	2.2	3.1	1.81	0.09	8.6	207
24	27	M	94/62	20	56	100	2.4	1.8	3.13	0.07	7.2	326
25	27	M	110/70	20	187	76	—	7.3	1.95	0.11	14.3	202
26	26	M	92/62	10	126	61	5.4	7.6	1.05	0.11	16.4	167
27	32	M	100/64	4	112	92	0	0.2	1.77	0.06	7.9	324
28	31	M	130/78	15	142	41	3.3	6.2	2.85	—	5.5	—
29	25	M	99/68	4	119	37	0.8	2.6	0.98	0.18	16.9	161
30	35	M	118/80	9	89	39	1.7	3.0	1.17	—	15.1	—
Diabetics with retinopathy (*denotes proliferative retinopathy)												
31*	30	M	115/76	15	107	115	0.9	1.8	2.07	0.15	8.0	136
32	22	F	107/74	15	94	132	1.2	4.5	5.18	0.35	3.4	57
33	24	M	150/102	19	117	56	1.6	4.6	4.04	—	3.5	—
34	30	F	104/73	14	158	55	3.2	3.6	2.92	0.16	7.3	140
35*	38	M	105/90	20	127	92	2.0	4.0	2.60	0.19	6.5	81
36	27	M	96/60	26	143	85	3.4	7.4	2.06	0.14	9.5	141
37	28	M	93/73	20	166	56	—	6.1	1.87	0.38	9.7	75
38	29	F	138/89	17	66	36	0.4	2.0	7.05	0.23	4.1	108
39	33	M	124/84	29	176	100	—	5.0	1.74	0.16	9.7	101
40	24	M	112/68	12	98	29	—	6.8	8.14	0.15	3.4	148
41	36	M	108/77	10	124	52	0.6	3.1	4.10	0.22	7.2	144
42	29	M	92/67	13	137	61	1.9	2.8	1.83	0.14	10.4	145
43	22	M	106/70	6	125	38	—	10.2	1.56	0.13	9.6	199
44	20	M	98/74	13	142	64	4.6	10.6	0.96	—	23.2	—
Patients not included—See text for explanation.												
Control												
45	21	F	102/68	—	131	121	—	2.0	1.60	—	12.4	—
Diabetics—no retinopathy												
46	24	F	92/61	<1	84	16	1.0	3.2	2.35	0.08	8.0	217
47	22	M	112/79	9	141	17	2.7	5.7	0.73	0.04	25.0	406
48	23	F	110/68	9	115	7	0.7	3.3	1.40	0.16	16.2	184
Diabetics with retinopathy												
49	30	M	108/63	9	—	—	2.6	2.1	1.25	0.11	16.6	240
50	30	M	110/68	17	—	—	0.7	3.3	2.31	0.13	7.6	195

TABLE 2

Clinical characteristics (mean ± 1 S.E.M.)

		Diabetics	
	Controls	No retinopathy	Retinopathy
Sex	5 M, 9 F	13 M, 3 F	11 M, 3 F
Age (yr.)	26 ± 1	29 ± 2	28 ± 2
Weight (kg.)	66 ± 3	68 ± 3	70 ± 4
Height (cm.)	170 ± 2	171 ± 2	171 ± 3
Duration of diabetes (yr.)	—	6 ± 2	16 ± 2 ($P < 0.001$)*
Blood pressure (mm. Hg)			
Diastolic	67 ± 2	70 ± 2	77 ± 3 ($P < 0.01$)*
Mean	79 ± 2	83 ± 2	88 ± 3
Pulse (beats/min.)	77 ± 2	84 ± 3	83 ± 3
Neuropathy (patients)	0	2	2

*Compared with controls.

45-50). Patient 45 (normal control) was found to be on oral contraceptives. Patients 46, 47, and 48 (diabetics without retinopathy) had 24-hour urinary sodiums of between 7 and 17 mEq., which suggested that their sodium intake was inappropriately restricted. Patients 49 and 50 (with retinopathy) had no 24-hour urinary data.

In nine additional patients, the pressor dose of norepinephrine was not recorded. In six of these patients the maximum appreciated blood pressure elevation was only 9 to 14 mm. Hg despite continued increase in the rate of norepinephrine infusion. One other patient developed a headache and another a bradycardia, thus necessitating discontinuation of the infusion. Another patient had no consistent changes in blood pressure.

Dose-response curves were constructed by standard linear regression technics[14] using the final blood pressure recordings (Y) at each dosage of pressor (X). The slope of this dose-response curve (b) is defined as the mm. Hg blood pressure rise per unit of infusate minus that amount of pressor used before any blood pressure rise. Since both the amount of pressor required to produce an initial increase in diastolic blood pressure and the rate of blood pressure increase varied from patient to patient, the amount of pressor required to produce a 20-mm. Hg increase in diastolic blood pressure was calculated for each patient for purposes of statistical comparison. This standardized dosage was calculated by solving the linear regression equation $Y = a + bX$ for X (ng./kg./min.) when Y = 20 mm. Hg.

Significant differences among the three groups were determined by analysis of variance followed by Student's *t*-test corrected for degrees of freedom to take into account the fact that all possible comparisons were being computed.[15]

Pearson's correlations[16] were done within each group to identify possible relationships between the variables.

RESULTS

The supine and upright plasma renin activity, the pressor dose of angiotensin II and of norepinephrine, and the slope of dose-response curves are given in table 1 and plotted in figures 1 and 2. Group mean values for plasma renin activity did not differ significantly (table 3).

For controls, diabetics without retinopathy, and diabetics with retinopathy, the pressor dose of angiotensin II (mean ± S.E.M.) was 11.5 ± 0.9, 12.9 ± 1.3, and 8.3 ± 1.3 and the slope was 2.0 ± 0.2, 1.6 ± 0.2, and 3.3 ± 0.6, respectively. Controls did not differ significantly from either diabetic group. Although the mean angiotensin II pressor dose was higher in diabetics without retinopathy than in those with retinopathy, the difference between means fell slightly short of statistical significance ($P = 0.059$). This lack of significance can be attributed to the inclusion of patient 44, with retinopathy, who required a high pressor dose. There was no obvious clinical reason to exclude this patient, so although exclusion could have been justified statistically, it was decided to utilize these data. Those with retinopathy had a significantly steeper slope than those without ($P < 0.02$), indicating, on the average, a greater sensitivity to angiotensin II.

TABLE 3

Laboratory characteristics (mean ± 1 S.E.M.)

		Diabetics	
	Controls	No retinopathy	Retinopathy
Creatinine (mg. %)	0.9 ± 0.04	0.9 ± 0.04	0.9 ± 0.05
Creatinine clearance (ml./min.)	118 ± 8	117 ± 7	127 ± 8
Blood glucose (mg. %)*	—	157 ± 17	169 ± 17
Hematocrit (%)	42 ± 1	43 ± 1	44 ± 1
Uric acid (mg. %)	4.9 ± 0.3	4.2 ± 0.3	4.7 ± 0.3
Cholesterol (mg. %)	170 ± 9	189 ± 12	173 ± 9
Serum Na (mEq./L.)	140 ± 1	139 ± 1	140 ± 1
K (mEq./L.)	4.1 ± 0.1	4.2 ± 0.1	4.3 ± 0.1
Urine Na (mEq./day)	92 ± 13	66 ± 8	69 ± 8
K (mEq./day)	64 ± 7	68 ± 5	69 ± 6
Plasma renin activity (ng./ml.)			
Supine	—	2.2 ± 0.5	2.0 ± 0.4
Upright	3.4 ± 0.3	4.6 ± 0.8	5.2 ± 0.7

*Mean of two or three determinations on day of studies.

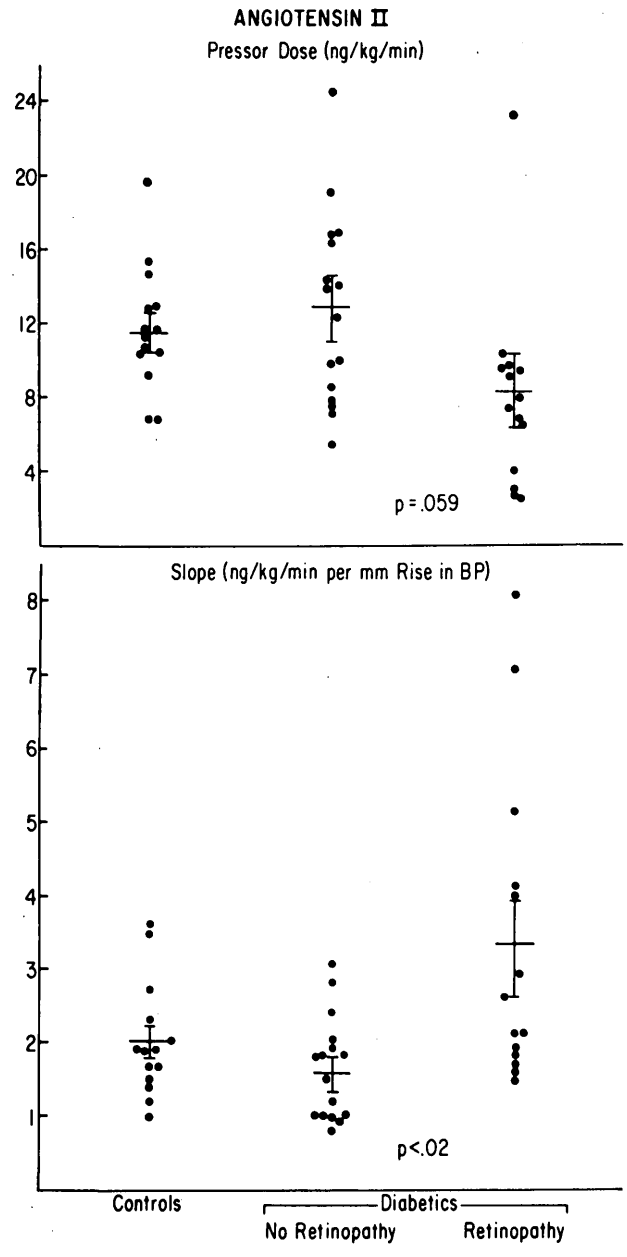

FIG. 1. Dose of angiotensin II (ng./kg./min.) producing a 20-mm. Hg increase in diastolic blood pressure (upper panel) and slope of the dose-response curve (ng./kg./min. per millimeter rise in blood pressure) in normal subjects, diabetics with no retinopathy, and diabetics with retinopathy (lower panel).

The pressor doses for norepinephrine were 163 ± 24, 212 ± 21, and 123 ± 11 and slopes 0.17 ± 0.03, 0.13 ± 0.02, and 0.20 ± 0.02 for each group, respectively. Differences between controls and either diabetic group were not statistically significant. Diabetics with retinopathy required a lower pressor dose than those without ($P < 0.006$). Additionally, the slope of the dose-response curve was steeper in those with retinopathy ($P = 0.05$).

No significant correlations were observed between the pressor doses or slopes of angiotensin II and norepinephrine and age, duration of diabetes, 24-hour urinary sodium, diastolic or mean blood pressure, or blood glucose. Although there was no correlation with

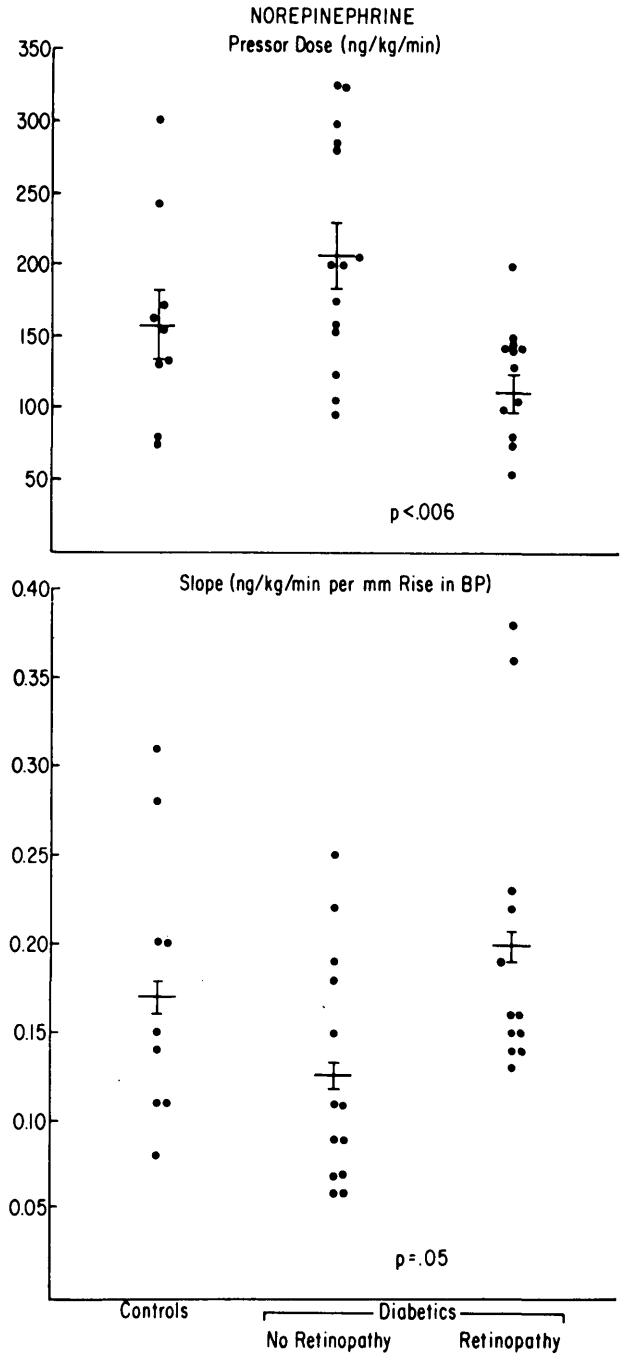

FIG. 2. Dose of norepinephrine (ng./kg./min.) producing a 20-mm. Hg increase in diastolic blood pressure (upper panel) and slope of the dose-response curve (ng./kg./min. per millimeter rise in blood pressure) in normal subjects, diabetics with no retinopathy, and diabetics with retinopathy (lower panel).

plasma renin activity in the control group, the angiotensin II pressor dose did correlate with upright renin activity in the diabetics without retinopathy ($r = 0.59$, $P < 0.05$). In those with retinopathy, the angiotensin II pressor dose correlated with supine renin activity ($r = 0.76$, $P < 0.05$) and upright renin activity ($r = 0.58$, $P < 0.05$).

DISCUSSION

These data suggest that, as a group, diabetics with retinopathy have increased vascular reactivity to both angiotensin II and norepinephrine when compared with diabetics with no evidence of diabetic complications. Neither diabetic group, however, was significantly different from normal subjects, who had a mean pressor responsiveness between that of the diabetic groups. One possible explanation for this latter observation is suggested by the observation that the normal subjects were in relative positive sodium balance compared with the two diabetic groups. A positive sodium balance has been reported to increase vascular reactivity in both man and animals.[17,18] Vascular reactivity, however, did not correlate with the 24-hour urinary sodium within any of the groups. Variations in the baseline blood pressure might offer another explanation. This is unlikely, however, since the diastolic pressure was significantly higher in diabetics with retinopathy (those most sensitive to vasopressors) than in the control group, with the diabetic group with no complications (those most resistant to vasopressors) having an intermediate level of diastolic blood pressure. In addition, neither the diastolic nor the mean pressure correlated with the pressor doses or slopes of the dose-response curves.

Increased vascular reactivity has also been observed in the experimental diabetic model. In the intact alloxan-diabetic rat, with polyuria secondary to glycosuria, angiotensin sensitivity is significantly increased whereas norepinephrine and tyramine sensitivity remain normal.[11] In perfused hindquarters of alloxan-diabetic rats, an increase in responsiveness to both angiotensin II and norepinephrine has been reported.[19] This difference between the intact animal and the isolated perfused vessels has not been explained. However, the finding of increased vascular reactivity in both human diabetics and the diabetic animal model suggests that the hyperresponsiveness may be due to common mechanisms associated with the diabetic state.

Several possible mechanisms might be invoked to explain enhanced vascular responsiveness in the diabetics with retinopathy. Differences in sodium balance, blood pressure, and blood glucose could each account for differences in vascular reactivity. However, no significant differences between the diabetic groups were noted with respect to any of these parameters. Other possible mechanisms include (1) nonspecific increases in vascular reactivity, (2) subnormal endogenous circulating levels of the respective pressor hormones, (3) differences in tissue-binding characteristics of the hormones, and (4) alterations in arteriolar luminal size.

Regarding the first possibility, the observation that enhanced vascular reactivity to both norepinephrine and angiotensin II is present in diabetics with retinopathy and in isolated perfused hindquarters of diabetic animals suggests that the response may be a nonspecific compensatory response to an as yet unknown stimulus associated with the diabetic state.

A relationship between circulating levels of vasopressors and the amount of vasopressor required for a given blood pressure response has been observed in both man and animals.[4,18] Further, in rats with alloxan diabetes, there is a highly significant correlation between the pressor dose of angiotensin II and the plasma renin activity.[11] In the currently reported diabetics, upright PRA did correlate with the pressor dose of angiotensin II in those without retinopathy and both supine and upright PRA correlated with the pressor dose in those with retinopathy, suggesting that angiotensin II sensitivity may be related to PRA. However, mean PRA did not differ significantly among the groups. Circulating angiotensin II was not determined. Variations in converting enzyme activity, renin substrate, and the hepatic clearance of renin[20] could individually or collectively account for alterations in circulating angiotensin II levels in the presence of normal levels of PRA. Further, there is evidence that catecholamines may be abnormal in diabetics with certain complications of their disease. For example, decreased plasma catecholamines have been reported in patients with long-duration diabetes and peripheral neuropathy.[10] Were decreased circulating levels of these vasopressors found in the present groups of diabetics, hyperresponsiveness to the hormones might be an expected response.

Increased responsiveness of peripheral vessels to norepinephrine could also result from impaired sympathetic innervation of these vessels, the phenomenon of denervation sensitization. Although signs of overt neuropathy were rarely observed in these patients,

subclinical neuropathy affecting the sympathetic nervous system could well be present.

The third possible mechanism for increased pressor sensitivity in diabetics with retinopathy could be variations in the tissue-binding characteristics of the respective hormones. Various data suggest that prior occupancy of vascular receptor sites by endogenous hormones will decrease pressor sensitivity to exogenous hormones.[21] Conversely, a decreased occupancy of sites could result in increased vascular reactivity.

The fourth mechanism might be anatomic abnormalities in the microvasculature. Hyalin thickening of arteriolar walls is prominent in diabetics with nephropathy and may be representative of a generalized vascular change. Although the group studied had no clinical evidence of renal disease, the observed retinopathy suggests that other abnormalities in the microvasculature are present. Such a change in resistance vessels would decrease the luminal diameter and could, with progression of the process, by itself result in increased blood pressure. Mild to moderate compromise of the luminal diameter may not result in elevated pressure under normal conditions. However, with small increments in humoral vasoconstrictor activity, the luminal diameter could be decreased sufficiently to result in marked increases in resistance to flow, thus elevating the blood pressure.

Any possible relationship between the observed alterations in vascular reactivity and the vascular complications of diabetes remains to be determined.

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