Renin, Angiotensin, and Norepinephrine in Alloxan Diabetes

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SUMMARY

The renin-angiotensin system and catecholamines were evaluated in short term alloxan diabetic rats which were either "severely diabetic" (polyuric with serum glucose > 450 mg. per 100 ml.) or "mildly diabetic" (normouric with serum glucose <450 mg. per 100 ml.). Plasma renin activity (PRA), renal renin activity (RRA), and the amount of angiotensin II producing an acute 10 mm Hg increase in blood pressure were all significantly decreased in the diabetic rats with this decrease correlating inversely with the severity of the diabetes. Insulin treatment of these rats resulted in partial normalization of angiotensin II responsiveness, PRA, and blood urea nitrogen. Injection of alloxan directly into the left renal artery did not decrease RRA. Protection of the left kidney during systemic alloxan injection did not prevent the decrease in renal renin activity. Streptozotocin-treated diabetic rats had increased vascular responsiveness to injected angiotensin II. Norepinephrine sensitivity (amount producing a 10 mm Hg increase in blood pressure) and norepinephrine stores (blood pressure response to tyramine) were normal in both alloxan- and streptozotocin-treated rats.

Blood volume as related to lean body mass was elevated in the severely diabetic rats thus suggesting one possible mechanism for the decrease in the renin-angiotensin system.

These results suggest that (1) norepinephrine responsiveness and norepinephrine stores are normal, and (2) there is suppression of RRA leading to decreased PRA and increased vascular reactivity to angiotensin II in the acutely diabetic alloxan-treated rat. Further, these abnormalities appear to be related to the degree of diabetes as assessed by serum glucose and twenty-four hour urine volume. DIABETES *23*:962-70, December, 1974.

Alterations in the activity of vasopressor systems have been observed in association with certain complications of diabetes mellitus. Circulating norepinephrine is increased in diabetics with poorly controlled diabetes or hypoglycemia[1] and decreased in those with evidence of neuropathy.[2] In diabetics with orthostatic hypotension, plasma renin activity is decreased and does not respond normally to the stimulus of upright posture.[3] Increased vascular responsiveness to angiotensin has been observed in the perfused hindquarters of alloxan diabetic rats[4] and to both angiotensin II and norepinephrine in diabetics with retinopathy suggesting that the circulating levels of these vasopressors are decreased.[5] Diabetic ketoacidosis may be associated with marked elevations of plasma renin activity, the renin response being blunted in patients with neuropathy.[6]

To assess the effect of abnormal carbohydrate metabolism on the renin-angiotensin system and on catecholamines in diabetic animals, alloxan-treated rats were studied. The data suggest that renal and plasma renin activity are decreased, that angiotensin II sensitivity is increased, and that norepinephrine and norepinephrine stores are normal in diabetic animals with diabetes for up to four months. This observed decrease in function of the renin-angiotensin system may be related to blood volume expansion and reversed by treatment with insulin.

METHODS

General Procedure—Rats given intravenous alloxan were followed for four to eighteen weeks. For one week prior to final studies, twenty-four-hour urine volumes were recorded. After withdrawing 3 ml. of

Presented in part at the 44th Scientific Session of the American Heart Association, Anaheim, California, November 1971; and abstracted in Circulation 44: Supplement II-93, 1971, and in Diabetes 21: Supplement I-354, 1972.

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Accepted for publication August 19, 1974.

blood (EDTA) for plasma renin activity determinations, each rat was transfused with 3 ml. of heparinized blood from a normal donor. Blood volumes and pressor responsiveness to angiotensin II, norepinephrine, and tyramine were then determined. Blood was then collected for the determination of glucose and blood urea nitrogen in the separated serum. The right kidney was frozen for determination of renal renin activity and the left kidney preserved in Bouin's solution for histologic examination.

All surgical procedures were performed with Nembutal (sodium pentobarbital) anesthesia 50 mg./kg. intraperitoneally.

Preparation of alloxan diabetic rats—Male albino Sprague-Dawley rats* weighing 160 to 260 gm. were fasted for eighteen hours following which alloxan,† 40 mg./kg., was given intravenously. The rats were then maintained on Purina Rat Chow (Ralston Purina, St. Louis) and tap water ad libitum.

Vascular responsiveness to pressor substances—Direct blood pressures were recorded from the right carotid artery on a Bird Kymograph (Phipps and Bird, Richmond) fitted with a palmer mercury manometer (Palmer Instruments, Cincinnati). The left jugular vein was catheterized for injections of synthetic α-L-asparaginyl^1-valyl^5 angiotensin II, norepinephrine, and tyramine using Micro Syringe-Burettes (California Laboratory Equipment, Berkeley) capable of delivering accurately 0.0001 ml. The dose response curve was assessed for each pressor agent in each rat and found to be linear to 15 mm Hg. The amount of pressor required to increase the blood pressure 10 mm Hg was then calculated. To insure pressor stability in each rat, norepinephrine was given initially, following angiotensin II, and again following tyramine injections. If the pressor response to norepinephrine varied more than 1 mm Hg, the pressor responsiveness was re-evaluated with each agent.

Glucose and blood urea nitrogen—No animals were fasted before these determinations. Serum glucose was determined using a ferricyanide AutoAnalyzer method and BUN using the diacetylmonoxime AutoAnalyzer method.

Plasma renin activity—(PRA)—was determined by radioimmunoassay using antibody against angiotensin I produced in rabbits. The antigen preparation has previously been reported.[7] This antibody did not react with renin substrate, angiotensin II, ACTH, lysine vasopressin, arginine vasopressin, oxytocin, or bradykinin.

With the following exceptions, the assay was performed as previously described.[3] For the generation of angiotensin I, 0.1 ml. of renin substrate (plasma from rats nephrectomized sixteen hours previously) was added to 0.5 ml. plasma. Angiotensin I generation without added substrate was linear for only ten minutes; with substrate for thirty to forty minutes. To insure that no substrate inhibition would occur, samples were incubated for ten minutes only. Reported values are corrected for dilution with substrate by multiplying by 1.2 and PRA is expressed as ng./ml./ten-minute incubation. Repeated assay of one sample gave a mean of 4.6 ± 0.3 (coefficient of variation 6 per cent), and of a second sample 1.5 ± 0.2 ng./ml. (coefficient of variation 13 per cent). Recoveries adding 50 pg. of angiotensin I to plasma ranged from 86 to 110 per cent. Recoveries adding 25 pg. ranged from 80 to 120 per cent.

To assess the validity of this radioimmunoassay for PRA under known experimental conditions, the PRA was determined in three groups of rats maintained on the following diet for seven days: 1) Normal rat chow with tap water (normal sodium diet), 2) A sodium free diet (General Biochemical, Chagrin Falls, Ohio) and tap water (low sodium diet), and 3) Normal rat chow with 0.9 per cent saline (high sodium diet). The mean PRA in rats fed the normal sodium diet was 7.0 ± 0.8 (S.E.M.), in rats fed the low sodium diet 13.0 ± 2.2 ($P < .01$), and in rats fed the high sodium diet 4.5 ± .6 ($P < .05$).

Renal renin activity (RRA)—was determined using the method of Boucher et al.[8] with modifications as described previously.[9]

Blood volumes—were determined using radioiodinated serum albumin (RISA) according to the method of Ormond et al.[10] with the following modifications. RISA was injected into the right carotid artery and the syringe was flushed four times with 0.4 ml. blood following which 0.4 ml. saline was injected. Blood samples were obtained at 5, 10, 15, and 20 minute intervals following the RISA injection and blood volume calculated from extrapolation to zero time.

Statistical analysis—Statistical differences between means in the parameters measured were determined by Student's paired or unpaired *t* tests.[11] Relationships between variables were analyzed using Pearson's correlation[12] and linear regression methods.[13]

*Charles River CD Strain Rats, Charles River Breeding Laboratories, Wilmington, Mass.

†Alloxan—Eastman Organic Chemicals, Distillation Products Industries, Rochester, N.Y.

RESULTS

Diabetes Mellitus in Alloxan Rats

The alloxan diabetic rats were divided into two groups on the basis of random serum glucose (figure 1). Of twenty-one rats with a serum glucose of less than 450 mg. per 100 ml., sixteen had normal twenty-four hour urine volumes. Therefore, alloxan diabetic rats with a serum glucose of less than 450 mg. per 100 ml. will be referred to as "mildly diabetic rats." By contrast, twenty-eight of twenty-nine rats with a serum glucose of more than 450 mg. per 100 ml. had twenty-four hour urine volumes greater than normal and will be referred to as "severely diabetic rats." In these rats, there was a direct correlation between the serum glucose and the twenty-four hour urine volume ($r = 0.49$, $P < .01$).

Blood Pressure Response to Pressor Agents

No significant difference in the amount of norepinephrine (figure 2) or of tyramine (figure 3) required to produce an acute increase in blood pressure of 10 mm Hg was observed among normal, mildly diabetic, or severely diabetic rats. Although there was

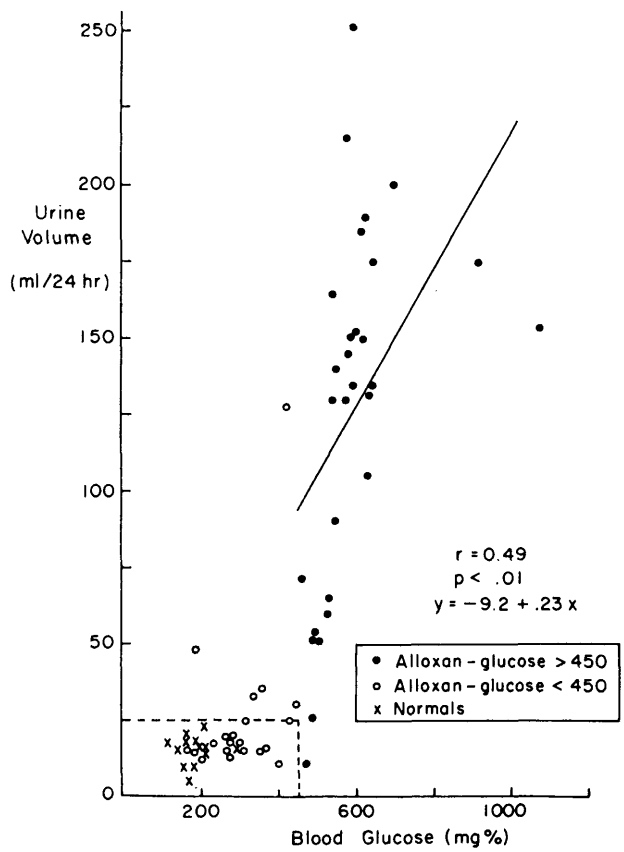

FIG. 1. Relationship between blood glucose and 24-hour urine volume in alloxan diabetic rats.

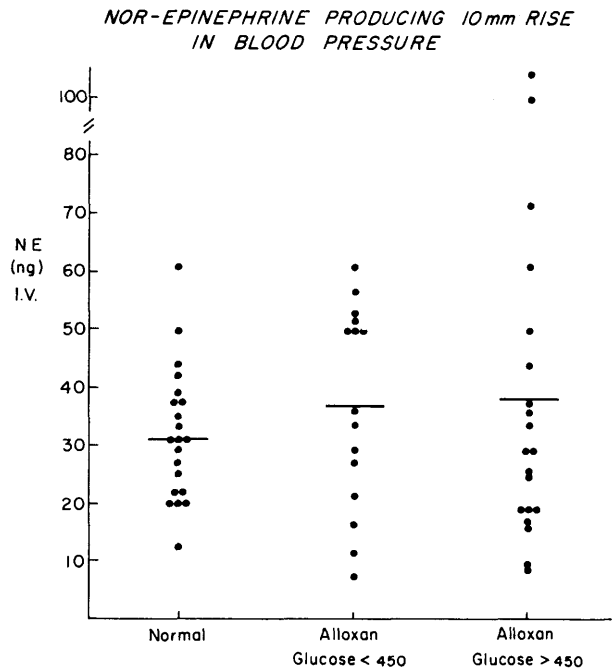

FIG. 2. Amount of norepinephrine required to acutely increase the blood pressure 10 mm Hg in normal, "mildly diabetic" and "severely diabetic" rats.

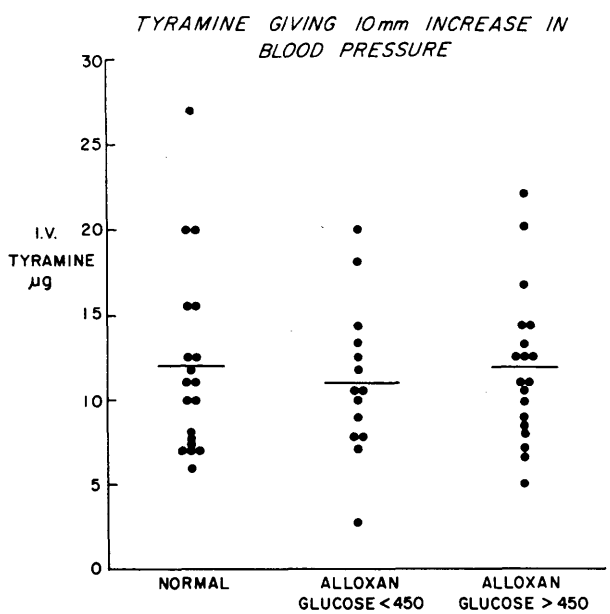

FIG. 3. Amount of tyramine required to acutely increase the blood pressure 10 mm Hg in normal, "mildly diabetic" and "severely diabetic" rats.

no significant difference in the pressor dose of angiotensin II between normal (mean 8.4 ng) and mildly diabetic rats (mean 10.1 ng), severely diabetic rats were significantly more sensitive to angiotensin II, requiring only 4.4 ng (mean) to increase the blood pressure 10 mm Hg ($P < .001$) (figure 4).

Weight, Hematocrit, and Blood Urea Nitrogen—Mean initial weights for control, mildly diabetic, and severely diabetic rats were similar, being 225, 236, and 228 gm. Mean terminal weights were 480 ± 17 (S.E.M.), 432 ± 31, and 311 ± 22 gm. Mean terminal hematocrits were 50, 51, and 46 per cent, respectively. Mean terminal BUN in control rats was 25 ± 1 mg. per cent, and in mildly diabetic rats 32 ± 3 mg. per cent ($P < .05$). In severely diabetic rats, the mean was 44 ± 4 mg. per cent, significantly different from controls ($P < .005$) and from mildly diabetic rats ($P < .05$). The BUN level correlated directly with the serum glucose in severely diabetic rats ($r = .47$, $P < .001$ in twenty-seven rats).

Plasma renin activity

Mean PRA for control rats was 6.7 ng/ml., significantly higher than mildly diabetic rats, 3.8 ng/ml. ($P < .01$). Severely diabetic rats had the lowest mean PRA, being 2 ng/ml., significantly less than control ($P < .001$) and mildly diabetic rats ($P < .05$) (figure 5).

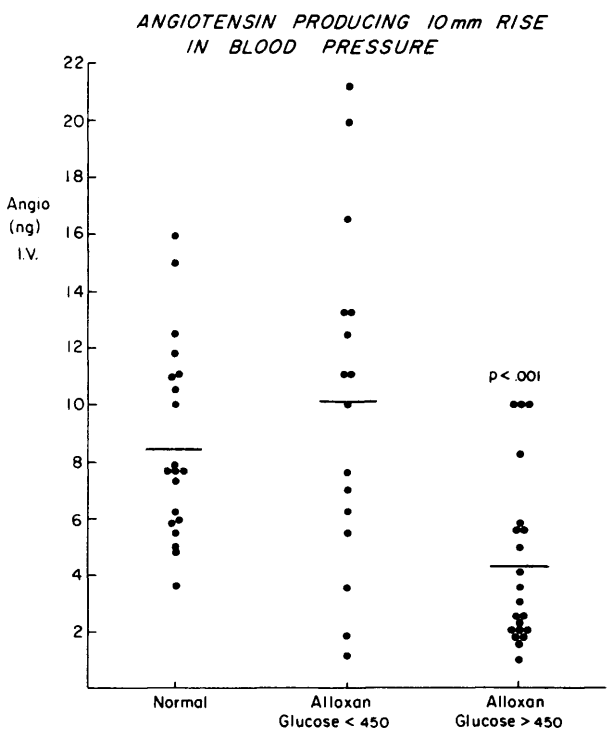

FIG. 4. Amount of angiotensin II required to acutely increase the blood pressure 10 mm Hg in normal, "mildly diabetic" and "severely diabetic" rats.

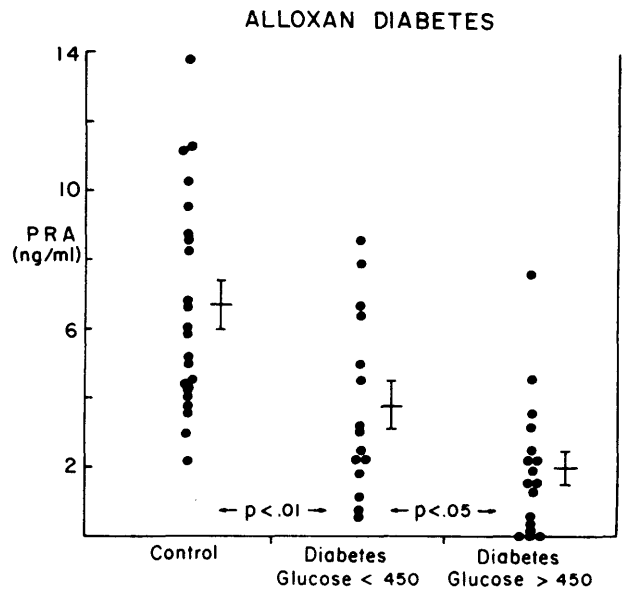

FIG. 5. Plasma renin activity in control, "mildly diabetic" and "severely diabetic" rats.

A significant correlation was observed between the amount of angiotensin II producing a 10 mm increase in blood pressure and the PRA ($P < .001$). There was an inverse correlation between PRA and urine volume ($P < .005$) and BUN ($P < .01$). There was no significant correlation between PRA and glucose.

Renal renin activity

RRA was determined in thirty alloxan diabetic rats and in ten controls. Diabetic rats (figure 6) had a mean RRA of 1,388 ng. per gram per minute, significantly lower than that of control rats which had a mean of 3,457 ng. per gram per minute ($P < .001$). Further, the RRA was significantly lower in severely diabetic (1,028 ng. per gram per minute), than in mildly diabetic rats (2,378 ng. per gram per minute) ($P < .005$).

An inverse correlation was observed between RRA and BUN ($P < .005$), urine volume ($P < .001$), and glucose ($P < .005$). Renal renin activity did not correlate with angiotensin sensitivity. PRA and RRA were simultaneously obtained in only six rats.

Blood Volumes

Blood volumes were determined in two series of

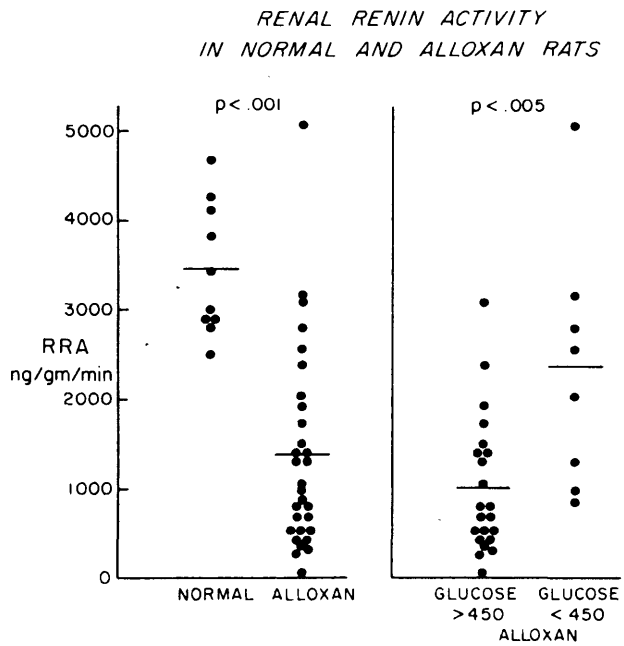

FIG. 6. Renal renin activity in normal and all alloxan rats (left), and in "severely diabetic" and "mildly diabetic" rats (right).

rats. In the first series, blood volumes in twenty-five diabetic rats were compared with those in thirty-one control rats. Severely diabetic rats (all with weights < 400 gm.) had a mean blood volume of 7.7 ± 0.1 ml. per 100 gm., significantly greater than a mean of 6.9 ± 0.2 ml. per 100 gm. observed in control rats of the same weight ($P < .01$). By contrast, mildly diabetic rats (weights > 400 gm.) had mean blood volumes similar to those of control rats of this weight.

To evaluate blood volumes in relation to lean body mass, a second series of thirty-six severely diabetic and thirty-six control rats pair-matched for weight were studied. Mean serum glucose was 616 ± 20 and 121 ± 7 ($P < .001$); mean blood urea nitrogen 36.6 ± 1.7 and 18.8 ± 0.8 ($P < .001$) in the two groups, respectively. Following determination of the blood volume, the rats were placed in fourteen 1-quart cylindrical cartons. The cartons were paired, one with diabetic rats of similar weights, the other with the same number of control rats with weights similar to that of the diabetic rats.

To determine total body potassium, which is an index of lean body mass, the ^{40}K counts in each carton were obtained in the total body counting facility at the Boston University Medical Center through the courtesy of Dr. Belton A. Burrows and Dr. Barry Genna. Table 1 summarizes these results. There was

TABLE 1

Weight, ^{40}K counts, and blood volume in cartons of diabetic and control rats*

No. Rats/Carton	Weight Grams/Carton		^{40}K Counts/Carton		Blood Volume ml./Carton	
	Diabetic	Control	Diabetic	Control	Diabetic	Control
4	1,301	1,302	9,227	9,082	85.7	85.2
6	1,218	1,322	8,718	9,854	96.0	82.9
5	1,268	1,276	9,187	9,553	97.2	86.1
5	1,653	1,640	11,920	11,959	111.8	104.7
5	1,485	1,521	10,167	10,788	101.8	96.2
5	1,359	1,390	9,427	9,584	96.2	92.7
6	1,436	1,426	9,939	10,140	108.7	92.5
Mean	1,388	1,411	9,798	10,137	99.6	91.5
	P = N.S.		P = N.S.		P < .01	

*See text for explanation

no significant difference in either ^{40}K counts per carton or rat weight per carton between the diabetic and control rats. By contrast, the total blood volume per carton of diabetic rats was significantly higher than that of control rats ($P < .01$).

^{40}K counts per rat were determined by the formula

$$\frac{\text{weight of rat}}{\text{total weight of rats per carton}} \times \text{total } ^{40}K \text{ counts per carton.}$$

The significance of group differences (diabetic vs. control groups) was then determined by analysis of covariance. Group means adjusted for counts per rat were 19.6 and 17.5 ml. per rat in each group, respectively ($P < .001$).

Insulin Treatment in Alloxan Diabetic Rats

Plasma renin activity, pressor responsiveness to angiotensin II and to norepinephrine, and BUN were determined in seventeen control, seventeen nontreated, and fifteen insulin-treated alloxan diabetic rats. Before treatment, mean urine volumes were 125 ml. and 119 ml., respectively, in the diabetic groups. In the insulin-treated group, NPH insulin (0.5 to 9 units) were given daily before noon according to the preceding twenty-four hour urine volume. Final studies were performed in the afternoon six weeks later. Table 2 summarizes these results. Partial control of the diabetes with insulin treatment was associated with a partial normalization of PRA and pressor responsiveness to angiotensin II. In each group, the pressor dose of angiotensin was inversely correlated with the serum glucose ($P < .05$).

Effect of Alloxan on Renal Renin Activity

In eight fasted rats, a 28-gauge needle was inserted

TABLE 2

Effect of insulin treatment in alloxan diabetic rats

	Control	Alloxan Diabetic Nontreated	Alloxan Diabetic Insulin-treated
24-hour urine volume (ml.)	19 ± 1	147 ± 7‡	36 ± 3†
Serum glucose (mg./100 ml.)	178 ± 11	622 ± 40‡	233 ± 52
Plasma renin activity (ng/ml.)	7.2 ± .6	4.8 ± .9*	6.2 ± 1
ng angiotensin II producing 10 mm Hg increase BP	10.6 ± 1.2	3.5 ± .4‡	6.1 ± 1.1*
ng norepinephrine producing 10 mm Hg increase BP	39 ± 4	35 ± 3	30 ± 3
Blood urea nitrogen (mg. %)	25 ± 1	43 ± 3‡	32 ± 4

* $P < .05$, † $P < .01$, ‡ $P < .001$ comparing nontreated and insulin treated alloxan diabetic rats with control rats.

TABLE 3

Streptozotocin-treated rats

	Control	Streptozotocin Treated	P-value
Plasma glucose (mg./100 ml.)	123 ± 13	462 ± 25	< .001
urine volume (ml./24 hrs.)	20 ± 2	196 ± 10	< .001
BUN (mg. %)	21 ± .8	28 ± 1.4	< .001
ng norepinephrine producing 10 mm Hg increase BP	18.0 ± 2.4	22.8 ± 2.6	NS
ng tyramine producing 10 mm Hg increase BP	11.6 ± .9	12.0 ± 1.1	NS
ng angiotensin II producing 10 mm Hg increase BP	5.3 ± .6	2.9 ± .2	< .002
Plasma renin activity (ng./ml.)	4.5 ± .7	2.7 ± .5	NS

across the aorta and into the left renal artery. Renal blood flow was maintained as evidenced by no ischemic discoloration of the kidney. Alloxan (3 mg./kg.) was injected over a five-second period. This dose represents approximately 8 per cent of the systemic diabetogenic dose of alloxan, i.e. the amount of a systemic dose reaching one kidney. Adequacy of renal arterial injection was shown by a transient ischemic discoloration of the kidney. Mean renal renin activity one month later in the injected (left) kidney was 2,629 ± 315 ng. per gram per minute and in the control (right) kidney 3,162 ± 361 (P = NS).

In eight other rats, that subsequently became severely diabetic, the left renal artery was occluded immediately before and for five minutes following the injection of alloxan. Mean RRA in the protected (left) kidney was 2,095 ± 339 ng. per gram per minute, significantly higher than 1,350 ± 363 in the unprotected kidneys ($P < .05$), but lower than normal kidneys (3,458 ± 237) ($P < .01$).

Streptozotocin-treated Rats

To evaluate the effect of a second diabetogenic agent on the renin angiotensin system, intravenous streptozotocin, 75 mg./kg. (obtained from William E. Dulin, Ph.D., The Upjohn Co., Kalamazoo, Mich.) was given to twelve nonfasted rats with ten nontreated rats as controls. Eight weeks later physiologic data were obtained (table 3). In these experiments, pentolinium tartrate (3 mg./kg.) was given in addition to the pentobarbital (50 mg./kg.). The amount of norepinephrine or tyramine required to produce a 10 mm Hg increase in blood pressure was similar between the groups. By contrast, streptozotocin-treated rats required 2.9 ± .2 ng angiotensin II, significantly less than control rats (5.3 ± .6 ng) to increase the blood pressure 10 mm Hg ($P < .002$). Although the PRA was less in the streptozotocin than in the control rats, the difference was not statistically significant.

Renal Pathology

Histologic examination of the kidneys of twenty-six rats (performed by Dr. Arthur A. Like) showed glomerular enlargement, tubular atrophy and dilatation, interstitial pyelonephritis, and tubular cell calcification. The severity of these changes correlated with the severity of the diabetes as assessed by the serum glucose. No changes consistent with diabetic glomerulosclerosis were observed in these rats.

DISCUSSION

The role of the renin-angiotensin system in normal and abnormal physiology is not yet defined. A major complication of human diabetes mellitus is the associated renal disease, often accompanied by significant hypertension. The possibility that alterations in the renin-angiotensin system might occur with diabetic nephropathy and be related to the accompanying "diabetic hypertension" and other diabetic complications has received little attention. To investigate the renin-angiotensin system in a diabetic animal model, the alloxan diabetic rat was chosen for study. In addition to data concerning this system, data were also collected concerning catecholamines. The strain of rats studied demonstrated a high renal threshold for glucose with significant polyuria associated with a serum glucose of more than 450 mg. per 100 ml. Therefore, on the basis of polyuria or normouria, the rats

were divided into "severely diabetic" or "mildly diabetic" rats. The severely diabetic rats had a significantly greater degree of renal disease as manifested by the blood urea nitrogen when compared with the mildly diabetic or control rats. These findings are important in the interpretation of the results of the renin-angiotensin studies.

The results of the present study suggest that a major alteration in the renin-angiotensin system is present in the alloxan diabetic rat. A consistent decrease in the activity of this system was observed by: (1) decreased renal renin activity, (2) decreased plasma renin activity, and (3) an increased vascular responsiveness to exogenous angiotensin II. A low pressor dose (amount of angiotensin II producing a 10 mm increase in blood pressure) is consistent with a decreased level of circulating angiotensin II.[7,14] A highly significant correlation was observed between PRA and "circulating angiotensin II" as assessed indirectly. That angiotensin II sensitivity did not correlate with RRA is not inconsistent in that variations in converting enzyme, renin substrate, and hepatic clearance of renin[15] may be present especially since alloxan can be hepatotoxic.[16] Each of these could affect angiotensin II concentrations. Too few rats had simultaneous PRA and RRA determinations to permit statistical analysis of these data.

The observation that all three parameters studied were decreased in these rats suggests that renal stores of renin are depleted primarily, with secondary decreases in PRA and angiotensin II. Although alterations in renin substrate may be present, such alterations would not result in decreased RRA determinations. Several possible explanations for decreased renal renin stores have been reviewed recently[17] and include (1) increased secretion of renin, (2) a direct toxic effect of alloxan on the juxtaglomerular apparatus, (3) a chronic suppression of renin synthesis, and (4) a metabolic defect in renin synthesis secondary to the "diabetic state." The first of these possibilities can be excluded in that plasma renin activity was also decreased in these rats.

Although histologic changes in the juxtaglomerular apparatus due to alloxan have not been described, it is well known that alloxan is toxic to the kidney. Glomeruli are histologically normal a short time after the injection of alloxan.[18] When one renal artery is occluded during the injection of alloxan and the kidneys are studied ten months later, mild to moderate glomerular changes are equally common in both the protected and the unprotected kidneys suggesting that glomerular lesions are not secondary to a direct toxic effect of alloxan.[18] By contrast, alloxan causes acute tubular degeneration during the first few days after injection.[19] In dogs, clamping one renal artery during alloxan injection protects the kidney from tubular atrophy and interstitial fibrosis in kidneys studied six to eight weeks later.[20] In rats with one renal artery clamped during and for five minutes after alloxan injection and studied ten months later, the tubular and interstitial lesions are present in both kidneys but more pronounced in the unprotected kidney.[18] These lesions are not observed in rats with both kidneys protected and studied five months after alloxan injection suggesting that the changes are secondary to the diabetes.[18]

To study the possibility of a toxic effect of alloxan on the juxtaglomerular apparatus, four studies were performed. In the first of these studies, the pressor dose of angiotensin II required to increase the blood pressure 10 mm Hg and the PRA were compared in insulin-treated and nontreated rats. It is clear that diabetic control was less than optimal in that the urine volume in treated rats was double that of control rats (a highly significant difference), but less than that in nontreated rats ($P < .001$). Despite this, the insulin-treated group required significantly more angiotensin than did the nontreated rats. Additionally, both the PRA and the BUN in insulin-treated rats (which in nontreated diabetic rats were significantly different from control rats) were similar to control rats. These results suggest that the compromise in renal function and the suppression of the renin-angiotensin system can both be reversed by partial control of the diabetes.

In a second study to evaluate a possible toxic effect of alloxan, another diabetogenic drug, streptozotocin, was employed.[16] Results, using this drug, were similar to that observed with the use of alloxan. Vascular reactivity to norepinephrine and to tyramine in streptozotocin-treated rats was similar to that observed in control rats, whereas vascular responsiveness to angiotensin II was significantly enhanced. The decreased dosage required for these pressor responses in this group of rats compared with alloxan-treated rats can be explained by the use of the ganglionic blocker, pentolinium, (which decreases renin release)[21] prior to final studies. The use of this drug may also account for the blunted PRA in these rats.

In the third of these studies, alloxan was injected directly into the left renal artery. The amount injected represented the fraction of a systemic diabetogenic dose reaching one kidney. Comparison of the renal renin activity between the injected and the control kidney showed no significant difference, suggesting

that alloxan itself is not toxic to the juxtaglomerular apparatus. The last study involved protecting the left kidney during the systemic injection of a diabetogenic dose of alloxan. These rats became severely diabetic. Despite this protection, the mean renal renin activity was 2,096 ng. per gram per minute, lower than in the kidneys of normal rats (3,458 ng. per gram per minute ($P < .01$). A further decrease in renal renin activity was observed in the nonprotected kidney. The results of these studies suggest that (a) alloxan alone does not decrease renal renin activity, (b) factors associated with the diabetic state do decrease renal renin activity, and (c) alloxan may have a "permissive" toxic effect in further decreasing renin when other factors pertaining to the diabetic state are operative.

A chronic suppression of renin synthesis secondary to some suppressive stimulus is a distinct possibility as the etiology of low renal renin activity in the diabetic rats. Only two of many such stimuli,[22] blood volume expansion and possible catecholamine depletion, were evaluated in the present study. With the marked serum hyperosmolarity, which could be expected with a serum glucose of 450 mg. per 100 ml. or above, one might anticipate an increase in plasma volume (providing adequate fluid intake to compensate for urinary losses) in an attempt to achieve osmotic equilibrium with the body cells. Also, the compromised renal function (elevated BUN) observed in these rats could be associated with hypervolemia. Indeed, blood volume measurements when related to body weight and to lean body mass as assessed by ^{40}K, were elevated in the severely diabetic rats and could explain the observed suppression of renin and angiotensin. In mildly diabetic rats, despite decreased RRA and PRA, blood volumes were similar to those observed in control rats. In mildly uncontrolled nonketotic human diabetics, with a mean serum glucose of 318 mg. per 100 ml., the blood volume although higher was not significantly different from that observed when the diabetes was controlled.[22] It is possible that small changes in blood volume, undetected by the method for blood volume determination employed, are present.

Much evidence has accumulated to suggest that catecholamines play a role in renin secretion (catecholamines stimulate renin release),[21,23] perhaps through a direct action on the juxtaglomerular cells.[24] Previously it was suggested that catecholamines are decreased in some diabetic patients after six years of diabetes.[25] Recently, measuring plasma levels, Christiansen[2] found decreased catecholamines in long-term diabetics with neuropathy. Therefore a decrease in catecholamines could be responsible for suppression of the renin-angiotensin system.

In these rats, however, both circulating norepinephrine and norepinephrine stores were normal as measured indirectly. The amount of norepinephrine required to produce a given pressor response was used as an indirect assessment of the circulating level of this hormone.[7,14] Likewise, the pressor response to tyramine was used to assess norepinephrine stores in nerve endings.[26]

It is unlikely, therefore, that alterations in catecholamines are responsible for the decreased renin in these rats. Although species differences may account for the discrepancy between man and the rats, it is possible that these "acutely" diabetic rats were not followed for a sufficient duration to observe alterations in catecholamines.

The fourth possibility for decreased function of the renin-angiotensin system in these diabetic rats would be a metabolic defect in the synthesis of renin, which in some way is associated with altered glucose metabolism. In man, diabetic ketoacidosis, a condition of acute insulin deficiency, plasma renin activity can be markedly elevated,[6] although there is no evidence that chronic insulin deficiency does not cause depletion of renal renin.

Regardless of the mechanisms involved in decreasing the activity of the renin-angiotensin system in the alloxan diabetic rat, the abnormalities are related significantly to the degree of diabetes mellitus. As there is evidence of renin deficiency in both the diabetic rat and some persons with diabetes, it appears that metabolic abnormalities associated with diabetes mellitus may result in a "renin-depleted state."

ACKNOWLEDGMENT

The author expresses appreciation to Mrs. Josefina Klose, Mrs. Andrea Taylor, and Miss Barbara Jacobs for their valuable technical assistance, to Dr. Arthur A. Like for assistance with pathologic examination of tissues, to Dr. Ray E. Gleason for assistance with statistical analysis of the data and to Drs. Belton A. Burrows and Barry Genna for their assistance in assessing lean body mass, and to Mrs. Della Donaldson for typing of the manuscript.

This work was supported by Public Health Service Grant HE 13368 and by the John A. Hartford Foundation Incorporated.

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