Renin-Angiotensin System in Phlorhizin Compared with Alloxan Diabetes in the Rat

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SUMMARY

In alloxan-treated diabetic rats, plasma renin activity (PRA) is decreased. One possible mechanism that may explain the decreased PRA is an increased delivery of sodium to the macula densa produced by the glucose osmotic diuresis, resulting in decreased renin release. To evaluate this possible mechanism, rats with phlorhizin diabetes, which produces a glucose osmotic diuresis without hyperglycemia, were studied and compared with rats with alloxan-induced diabetes. Whereas phlorhizin-treated rats had low blood glucose and alloxan-treated rats had elevated glucose, the glucose osmotic diuresis was similar in the two groups. PRA and plasma renin concentration (PRC) were significantly increased in the phlorhizin group. In the alloxan group, PRA was decreased and angiotensin II sensitivity increased, both significantly. Plasma renin substrate (PRS) remained adequate in each group. These results suggest that the decreased PRA in alloxan-induced diabetes is due neither to factors associated with the glucose osmotic diuresis including changes in renal tubular sodium nor to decreased PRS.

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Previous studies have shown that plasma renin activity (PRA) is decreased and vascular reactivity to angiotensin II is increased in alloxan- and streptozotocin-induced diabetes in the rat.[1] In the human with diabetes, PRA and aldosterone are normally responsive in the absence of diabetic complications but poorly responsive when the diabetes is complicated by neuropathy or nephropathy.[2,3] The mechanisms responsible for decreased PRA in diabetes have not been clearly defined. In both the rat with chemically induced diabetes and in the human, hyperglycemia can produce extracellular hyperosmolarity with subsequent volume expansion suppressing renin release.[1,4] Hyalinization of the afferent arteriole adjacent to the juxtaglomerular cells or invading the juxtaglomerular cells may block renin release to the circulation or destroy the juxtaglomerular cells completely in the human.[5,6] Further, increased amounts of circulating pro-renin have been observed in patients with nephropathy, which suggests inadequate conversion of pro-renin to renin.[7,8] Finally, catecholamines may be depleted, thus decreasing catecholamine stimulation of renin release.[9]

Another possible mechanism that may decrease renin release could be a change in renal tubular sodium produced by the osmotic diuresis of glucose or the glucose osmotic diuresis itself. To study this, it is imperative to produce the glucose osmotic diuresis in the absence of hyperglycemia to eliminate mechanisms dependent on the hyperglycemia. In the present report, the renin-angiotensin system and electrolyte balance are studied in rats treated with phlorhizin, which produces a glucose osmotic diuresis without hyperglycemia. Results are compared with those in rats with alloxan-induced diabetes having a similar glucose osmotic diuresis.

MATERIALS AND METHODS

Four groups of male, albino Sprague-Dawley rats weighing 150–200 g (Charles River CD strain rats, Charles River Breeding Laboratories, Wilmington, Massachusetts) were maintained in individual metabolic cages for 18 days. Group 1 (phlorhizin control) consisted of 11 rats given 0.2 ml propylene glycol s.c. three times daily. Group 2 (phlorhizin) consisted of 11 rats receiving 120 mg of phlorhizin (Lot no. 17793, ICN Pharmaceuticals, Life Sciences Group, Plainville, New Jersey) in 0.2 ml propylene glycol s.c. three times daily (total daily dose 360 mg). Group 3 (alloxan control) consisted of eight rats given 0.2 ml normal saline i.v. after an 18-h fast. Group 4 (alloxan) consisted of 18 rats treated with alloxan (Eastman Organic Chemicals, Distillation Products Industries, Rochester, New York) 40 mg/kg i.v. after an 18-h fast. All rats were maintained on Purina

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TABLE 1
Weight, urine volume, and laboratory determinations in the four groups of rats

	Group 1		Group 2	Group 3		Group 4
	Phlorhizin control	P	Phlorhizin	Alloxan control	P	Alloxan
Weight (g)	339 ± 5	<0.001	283 ± 7	298 ± 3	<0.001	231 ± 8
Glucose (mg/dl)	159 ± 11	<0.025	127 ± 6	156 ± 8	<0.001	516 ± 24
BUN (mg/dl)	22 ± 1	NS	29 ± 4	20 ± 1	<0.001	37 ± 2
Urine						
Volume (ml/day)	21 ± 2	<0.001	69 ± 2	25 ± 2	<0.001	86 ± 5
Glucose (g/day)	0	<0.001	2.9 ± 0.1	0	<0.001	2.6 ± 0.4
Protein (mg/dl)	9 ± 3	<0.01	24 ± 4	20 ± 8	<0.02	57 ± 8

Rat Chow (Ralston Purina, St. Louis, Missouri) and distilled water ad libidum. Individual 24-h urinary output was collected in each rat of each group. Daily fluid and food intake was determined in groups 1 and 2.

After 18 days in metabolic cages, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Direct blood pressures were recorded from the right carotid artery by using a Bird kymograph (Phipps and Bird, Richmond, Virginia) fitted with a Palmer mercury manometer (Palmer Instruments, Cincinnati, Ohio). The left jugular vein was catheterized for injections of synthetic alpha-L-asparaginyl-valyl[5] angiotensin II by using Micro-syringe-burettes (California Laboratory Equipment, Berkeley, California) capable of delivering accurately 0.0001 ml. Linearity of dose-response curves was assessed for the pressor in each rat. The amount of pressor required to increase the blood pressure 10 mm Hg was recorded.

After this procedure, blood was obtained for the determination of glucose, BUN, PRA, plasma renin concentration (PRC), and plasma renin substrate (PRS).

Daily sodium and potassium intake was calculated from the daily food intake, which contained 0.45% Na and 0.92% K/g. Daily urinary Na and K levels were determined in groups 1 and 2 by flame photometry. Serum glucose (nonfasting) was determined by using the ferricyanide autoanalyzer method and BUN by using the diacetyl monoximine autoanalyzer method. PRA was determined as previously described[2] and modified for rat plasma.[1] Plasma renin concentration and PRS were determined as previously described[10] by using plasma from rats nephrectomized 24 h before for the substrate and rat kidney extract for the renin. The only modification to this method was the use of Tris acetate buffer (pH 7.4) containing 1 mg/ml of bovine serum albumin.[11]

The results were expressed as mean ± SEM for all parameters studied. Statistical differences between means were determined by Student's *t* test.

RESULTS

Results are shown in Tables 1, 2, and 3. Similarities between phlorhizin- and alloxan-treated rats, when compared with control rats, included a failure to gain weight normally, an increase in urine volume, and an increase in urinary glucose and protein excretion (Table 1). The blood glucose was decreased in the phlorhizin- and increased in the alloxan-treated rats.

Table 2 shows the Na and K intake and output for control and study days in phlorhizin control and phlorhizin-treated rats. Although intake of Na and K was similar in the two groups, urinary losses of each cation were increased in the phlorhizin-treated rats, resulting in a negative Na balance over the study period. In alloxan-treated rats studied under similar conditions, negative Na and K balance has been observed also.[11]

The results of PRA, PRC, PRS, and the pressor dose of angiotensin II required to increase the blood pressure 10 mm Hg are seen in Table 3. Whereas alloxan-treated rats, when compared with controls, had decreased PRA ($P < 0.001$), the phlorhizin-treated rats when compared with their controls had elevated PRA ($P < 0.02$). PRC followed the same pattern with a decrease that was not statistically significant in the alloxan-treated rats and a significant increase in the phlorhizin-treated rats when compared with their controls ($P < 0.05$). PRS decreased in both groups, with the decrease meeting statistical significance only in phlorhizin-treated rats ($P < 0.005$). The pressor dose of angiotensin II was decreased in alloxan-treated rats compared with their controls ($P < 0.005$), which is consistent with increased pressor responsiveness. The difference in pressor dose in phlorhizin-treated rats compared with controls was not statistically significant.

DISCUSSION

Alterations in the Na load in the renal tubule reaching the region of the macula densa are known to effect renin release.[12–14] Alloxan-induced diabetes in the rat produces glycosuria with an associated water and Na loss from the kid-

TABLE 2
Sodium and potassium balance on control day and during 18 study days ($\bar{X}$ ± SEM) in control and phlorhizin-treated rats

	Control day		Study days		
	Group 1	Group 2	Group 3		Group 4
	Phlorhizin control	Phlorhizin	Phlorhizin control	P	Phlorhizin
Sodium (mEq)					
Intake/day	5.9 ± .2	6.0 ± .4	6.3 ± .1		6.4 ± .1
Urine/day	2.3 ± .3	3.5 ± .8	1.9 ± .1	<0.001	3.4 ± .1
Balance	3.8 ± .3	2.5 ± .8	4.4 ± .1	<0.001	3.1 ± .1
Potassium (mEq)					
Intake/day	7.2 ± .2	7.3 ± .5	7.6 ± .1		7.8 ± .1
Urine/day	3.3 ± .4	4.7 ± .8	2.9 ± .1	<0.001	4.4 ± .1
Balance	4.0 ± .4	2.5 ± 1.0	4.7 ± .1	<0.001	3.4 ± .1

TABLE 3
Renin determinations and pressor dose of angiotensin II in the four groups of rats

	Group 1		Group 2	Group 3		Group 4
	Phlorhizin control	P	Phlorhizin	Alloxan control	P	Alloxan
PRA (ng/ml)	86 ± 7	<0.02	149 ± 23	113 ± 6	<0.001	49 ± 5
PRC	325 ± 27	<0.05	992 ± 285	307 ± 28	NS	209 ± 32
PRS (ng/ml)	929 ± 33	<0.005	628 ± 81	911 ± 56	NS	758 ± 55
Pressor dose of angiotensin*	16 ± 3	NS	25 ± 5	17 ± 2	<0.005	10 ± 1

* 1 ng to increase blood pressure 10 mm Hg.

neys. Since PRA is decreased in this model, one must question if the decreased PRA is secondary to changes in renal tubular Na produced by the glucose osmotic diuresis.

Phlorhizin has the unique characteristic of producing a renal glucose osmotic diuresis without hyperglycemia. The chemical and physiologic properties of phlorhizin have previously been described.[15] It is, therefore, an ideal agent with which to evaluate the effect of an isolated glucose osmotic diuresis on other physiologic parameters including the renin-angiotensin system.

The results of the present study suggest that although the plasma glucose is elevated in alloxan-induced diabetes and decreased in phlorhizin diabetes, there are several similarities between these two models. Both failed to gain weight appropriately, both had increases in BUN, and both had a glucose osmotic diuresis accompanied by increased Na, K, and protein excretion. The comparatively greater increase in BUN in the alloxan model may be accounted for by increased tissue protein breakdown. The increase in sodium excretion in both models is consistent with an increase in the delivery of sodium to the region of the macula densa in the renal tubule.

If the sodium load reaching the macula densa in the alloxan diabetic rat model was responsible for the decreased PRA and PRC, then a decrease in PRA and PRC in the phlorhizin model would be expected also. However, this did not occur. In fact, in the phlorhizin model, which had a glucose osmotic diuresis similar to that in the alloxan model, the PRA and PRC actually increased. These findings suggest that the mechanism of decreased renin in alloxan-treated diabetic rats is not secondary to tubular sodium changes produced by the glucose diuresis or to the glucose osmotic diuresis itself.

One possible explanation for the difference in PRA and PRC levels between these two models would be differences in plasma volume due to the level of extracellular glucose. Previously, alloxan diabetic rats with hyperglycemia have been shown to have elevated blood volumes.[1] This can be explained by the fact that the high blood glucose produces an osmotic load in the extracellular space, and, to achieve osmotic equilibrium between the intracellular and extracellular space including the plasma, the extracellular space must expand. In the phlorhizin model, the blood glucose was decreased. This decreased extracellular glucose could decrease the osmotic load in the extracellular space and could then result in a decreased plasma volume that would serve as a stimulus for renin secretion and explain the observed increase in PRA and PRC.

The circulating level of angiotensin II should be increased with elevated PRA and decreased with low PRA. An indirect assessment of the circulating level of angiotensin II can be obtained by determining the amount of angiotensin II necessary to produce a given increase in blood pressure. If circulating angiotensin II is high, the animal will be tachyphylactic to infused angiotensin II and vice versa. In this study, alloxan diabetic rats required a low dose of angiotensin II to increase the blood pressure 10 mm Hg, suggesting that the low PRA is associated with low angiotensin II in this model. Although the phlorhizin rats required a higher dose of angiotensin II to increase the blood pressure 10 mm Hg, consistent with increased circulating levels of angiotensin II, the difference was not statistically significant.

Another possible mechanism that may explain the decrease in PRA in the alloxan diabetic model can be excluded by this study. Renin substrate is produced in the liver. As alloxan is hepatotoxic, it theoretically could produce liver damage and compromise the formation of renin substrate. However, this did not occur. Both the alloxan and the phlorhizin models had sufficient substrate for the renin-renin substrate reaction to achieve completion.

In summary, these results suggest that the decreased PRA in the alloxan diabetic rat model is not the result of factors associated with a glucose osmotic diuresis. Further, the decreased PRA cannot be explained by a decrease in renin substrate. Plasma volume changes produced by varying levels of extracellular glucose can be postulated to explain the observed changes in PRA in each of these models.

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