Short Report

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Serum Antiotensin Converting Enzyme Activity in Streptozotocin-induced Diabetic Rats

It is well recognized that diabetes mellitus is associated with an increase in kidney size, in both humans and experimental animals (1,2). The morphologic changes that occur in diabetic organs have been best characterized in the kidney. Early in the course of diabetes, kidney cells undergo hypertrophy and, later on, hyperplasia (3). Recent evidence indicates a possible role for the renin-angiotensin system (RAS) in the pathogenesis of glomerular injury in diabetes mellitus (4). RAS has important hemodynamic and growth-enhancing properties, which may play role in the development of diabetic microvascular complications (5). However, in the diabetic human and rat, the results of studies on the activity of the serum angiotensin converting enzyme (ACE), the major enzyme regulating angiotensin II production, have been conflicting. ACE activity, in both humans and rats has been reported as being decreased (6, 7), normal (8, 4) or even elevated (9, 10, 11). Therefore, the present study was designed to clarify the direct effect of diabetes on serum ACE activity in streptozotocin (STZ) -induced rats.

Male albino rats weigting 150-200 g were used in the present investigation. The experimental group (n=7) was injected (i.p.) with STZ (freshly dissolved in citrate buffer, pH 4.5, 55 mg/kg), while the control group (n=10) was injected with buffer only. All rats had free access to food and water for 5 weeks. There after, control and diabetic rats were sacrificed by rapid decapitation.

Body weights were obtained before treatment and prior to sacrifice. Blood samples were collected from the tail vein at the time of sacrifice and blood glucose levels were determined using an Ames glucometer (Miles Laboratories Inc., Elkhart, Ind, USA).

Serum ACE activity was determined according to the method of Holmquist (12) by Sigma diagnostic kit (Sigma Diagnostics St. Louis, MO, USA) in Technicon RA-XT autoanalyzer (Miles Inc. Tarrytown, NY, USA). Hydrolysis of synthetic tripeptide substrate N-[3-(2-furyl)acryloyl]-L-phenylalanylglycglycine (FAPGG) by ACE results in a decrease in absorbance at 340 nm. ACE activity in the samples is determined by comparing the sample reaction rate to that obtained with the ACE calibrator. One unit ACE activity is defined as the amount of enzyme that will catalyze formation of one micromole of furylacryloylphenylalanine (FAP) per minute under the conditions of assay.

In the present study, after STZ treatment, rats demonstrated polyphagia, polydipsia, polyuria and stable hyperglycemia for 5 weeks (data not shown).

Body weight measurements before treatment and at the time of sacrifice revealed a significant difference in the body weight of STZ-treated rats relative to controls (Table 1). Furthermore, blood glucose determinations showed a significant hyperglycemia relative to control animals. Serum ACE activity in diabetic rats was 337.9 ± 46.5 U\L (range 288-420) and significantly increased by 57% compared to the value of 193.7 ± 98.2 U\L (range 44-303), $p < 0.002$. Table 1 summarizes the changes in body weight, blood glucose levels and serum ACE activity 5 weeks after STZ treatment. Results were expressed as mean± SD and student's t-test was used for comparison of the two groups.

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	Body weight (g)	Blood glucose (mg/dl)	Serum ACE activity (U/L)
Controls (n=10)	228.75±37.58	223.0±105.9	193.7±98.2
Diabetes (n=7)	154.0±35.7[*]	382.5±40.2[‡]	337.9±46.5[f]

Table 1. Changes in the body weights, blood glucose levels and serum ACE activities (mean± SD) of control and diabetic rats.

[*]$p<0.005$, [‡]$p<0.001$, [f]$p<0.002$ significance relative to control.

Diabetes mellitus is associated with diffuse vascular damage in vascular beds which, as has been suggested, may either contribute to or be caused by the alterations of RAS in the circulation (5). Vascular reactivity to exogenous angiotensin II may be altered in the early stages of diabetes mellitus, reflecting either a receptor associated event or altered post receptor physiology (13). In the present study, serum ACE activity is significantly higher in diabetic animals than in controls, and this finding is similar to those reported by several groups in the clinical situation.

Previous observations have shown the growing association of vascular hypertrophy with increased tissue ACE concentration (5). A universal underlying abnormality in the pathogenesis of hypertension, atheroscleorosis, myocardial dysfunction and diabetic glomeruloscleorosis involves alteration in smooth muscle cell structure, function and growth. Angiotensin II, through its effects on contractility, growth and the sympathetic nervous system, may potentially play a key role in this pathologic process and thus contribute to the development of these cardiovascular and renal complications of diabetes mellitus. ACE inhibitors and some direct renin inhibitors prevent or slow the progression of some of these complications, which further suggests a pathologic role for the RAS in diabetes mellitus (14). Plasma ACE activity has been linked to diabetic microvascular disease, including microalbuminuria and diabetic retinopathy (15, 9). However, other authors detected no such correlation with vascular disease, the degree of metabolic control, or with the type of diabetes (5). In addition, the results of ACE activity in diabetic humans have been conflicting (6, 8, 9, 16).

In the rat tissue, ACE activity has been found in the kidney, lung, blood vessels, brain and ovary (17). It was proposed that the lung enzyme is released into the circulation, whereas the renal enzyme is excreted into the urine (10). Serum ACE activity in diabetic rats has been reported as being decreased (7), normal (4) or even elevated (10, 11). The STZ induced diabetic rat model is similar to humans, since these rats develop hypertension, polyuria and renal hipertrophy. In the present study, serum ACE activity in the diabetic rat was found to be elevated after 5 weeks of STZ-administration. This is in agreement with the finding of Velentovic et al. (11), showing that serum ACE activity in the diabetic rats increased 58% beginning 12 days post STZ treatment (55 mg/kg) and this increase continued for 7 weeks post STZ treatment. In the same study serum ACE activity was reported to be unchanged 3 days post STZ administration. Supporting these findings, Erman et al. (10) reported that serum ACE activity was elevated 11 and 14 days after STZ administration, but not 3 and 7 days, suggesting that STZ does not have a direct immediate effect on ACE. However, more recent reports do not confirm those findings of increased ACE activity in diabetic rats. Anderson et al. (4) have reported that serum ACE activity was normal in diabetic rats after 8 weeks of STZ administration in a similar concentration that was used in the present and the aforementioned studies. Furthermore, in male Zucker fatty diabetic rats, used as models of non-insulin-dependent diabetes mellitus, serum ACE activity. It was found to be reduced (7). It remains unclear whether the above mentioned differences arise from the differences in the methods applied in measuring ACE. It is worthwhile to note the previously reported rapid decay of high ACE activity of properly frozen sample within only a few days after collection, thus confirming the need for prompt analysis of the samples for an accurate measurement of specimens with high ACE content (8).

In conclusion, in the present study we clearly demonstrate that serum ACE activity is increased in the STZ-induced rats. Although it has been speculated that diffuse vascular damage in diabetes mellitus may release ACE into the blood stream, the precise underlying mechanism for the increased serum ACE activity in diabetes mellitus remains to be clarified.

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