Plasma Inactive Renin in Patients with Diabetes Mellitus: Effects of Standing and the Relation to Serum Protease Inhibitor

NOBUO SHIMOJO, SATORU FUJII, YOSHIHIKO FUNAE AND MASAHISA WADA

Second Department of Internal Medicine, Laboratory of Chemistry, Osaka City University Medical School, Osaka, 545

Abstract

In order to investigate the mechanisms of increased plasma inactive renin in diabetics with diabetic microvascular complications, changes in active and inactive renin with the progress of diabetes mellitus were studied, and effects of standing on inactive renin release and the relationship between plasma inactive renin and serum trypsin or protease inhibitors were also studied. Inactive renin increased with the aggravation of diabetes mellitus, but active renin didn't show significant changes with the aggravation of diabetes mellitus. Active renin was significantly increased both in the healthy subjects and in the diabetic patients when they were in an upright position, but no significant change was observed in inactive renin. Serum trypsin in diabetics with retinopathy and nephropathy was lower than that in those with no clinical sign of microangiopathy, but the correlation between plasma inactive renin and serum trypsin was not significant. There was a significant correlation between plasma inactive renin and serum α2-globulin (r=0.52, p<0.01). Although plasma inactive renin was not significantly correlated with serum α1-antitrypsin, there was a significant correlation between plasma inactive renin and serum α2-macroglobulin (r=0.61, p<0.01).

These results show that the increased levels of plasma inactive renin observed with the development of diabetic microangiopathy are probably related to the altered plasma protein metabolism observed in patients with diabetes mellitus. However, it is not clear whether this altered protein metabolism is related to the conversion from inactive to active renin.

Alterations in the renin-angiotensin-aldosterone system have been reported in patients with diabetes mellitus, especially in those with microvascular complications (Christlieb et al., 1974, 1976; Burden and Thurston, 1979). Day et al. (1975a, b) found an inactive form of renin of a high molecular weight in the plasma of patients with diabetic nephropathy. We reported that increased acid activatable inactive renin in the plasma of diabetes mellitus might be related to the progression of diabetic microangiopathy (Fujii et al., 1980) and that acid activation was not associated with conversion from a high molecular form to a low molecular form of renin (Shimojo et al., 1981). High inactive renin levels in the plasma of diabetic nephropathy suggest the following possibilities: an increase in synthesis, a decrease in release or a defect in activation. Although details of the mechanisms of activation of inactive renin are still ill-defined, serine proteases are known to play an important role in this activation (Atras et al., 1978; Derkx et al.,

Received October 23, 1982
The present studies were carried out to investigate the release of inactive renin after the stimulation of standing. We then measured serum trypsin and α1-antitrypsin and α2-macroglobulin in order to evaluate the relationship between plasma inactive renin and the serum protease of trypsin or protease inhibitors.

**Material and Methods**

**Subjects**

Seven healthy Japanese subjects (mean age, 36.5±3.9 yr) and 25 patients with diabetes mellitus from the outpatient clinic in Osaka City University Hospital were studied. The 25 diabetics were classified into three groups: 10 diabetics with no diabetic retinopathy or proteinuria (Group I; mean age, 50.4±3.2 yr), 7 diabetics with retinopathy (Scott I-IIa) and proteinuria of less than 30 mg/dl (Group II; mean age, 58.1±3.2 yr) and 8 diabetics with retinopathy (Scott IIb-V) and clinical nephropathy as defined by persistant proteinuria at least 300 mg/dl (Group III; mean age, 52.6±3.6 yr). Each subject was on a liberal sodium intake while off all antihypertensive medication for at least one week.

**Methods**

After an overnight fast, each subject rested in a supine position for at least one hour and blood samples were collected before and after the subjects had been upright for one hour. Blood samples were drawn into chilled EDTA tubes, centrifuged at 4°C and 3000 rpm for 20 minutes and the plasma was aspirated. Plasma renin activity (PRA) was measured by radioimmunoassay using a commercial kit (CEA-IRE-SORIN, Italy). 2, 3-Dimercaptopropanol (BAL) and 8-hydroxyquinoline were added to all studies as angiotensinase inhibitors. Activation of inactive renin was performed by the procedures of acidification described by Weinberger et al. (1977); plasma was dialyzed against 0.05 M glycine-HCl buffer containing 0.1 M NaCl, pH 3.3, for 24 hours at 4°C and then dialyzed against 0.05 M phosphate buffer containing 0.1 M NaCl, pH 7.4, for an additional 24 hours. The angiotensin I generated in the incubation of non-acidified plasma, which had been dialyzed against pH 7.4 buffer for 48 hours at 4°C, with pig renin substrate (Pentex, 1400 ng angiotensin I equivalent per ml of incubation medium) was expressed as plasma renin concentration (PRC). The angiotensin I of acidified plasma was expressed as the total renin concentration (TRC) which includes both active and inactive renin. The inactive renin concentration (IRC) was calculated as TRC minus PRC. Serum trypsin was measured by radioimmunoassay (Hochst) (Temler and Felber, 1971). The biuret method was used for the determination of serum total protein and serum α2-globulin was determined by means of cellulose acetate electrophoresis (Kohn, 1958). In assays of serum α1-antitrypsin and α2-macroglobulin, the single radial immunodiffusion technique was used (Mancini et al., 1965).

Student's t-test was used to evaluate the statistical significance.

**Results**

Table 1. shows PRA, PRC and IRC in each group before and after being upright. PRA and PRC increased significantly in all groups after being upright for one hour (p<0.05, respectively) but no significant change was observed in IRC.

The mean values for serum trypsin were 280.2±28.9 ng/ml in the healthy subjects, 299.6±24.7 ng/ml in Group I, 195.7±29.4 ng/ml in Group II and 180.1±17.2 ng/ml in Group III diabetics, respectively. Although there was no significant difference in serum trypsin between the healthy subjects and Group I diabetics, serum trypsin levels in

| Table 1. Effects of standing on active and inactive renin in diabetics and controls |
|-----------------------------------|---------|------------|---------|--------|---------|
|                                  | PRA     | PRC        | IRC     |
|                                  | Recumbent| Standing   | Recumbent| Standing| Recumbent| Standing|
| Controls                         | 7       | 2.3±1.0    | 8.8±3.0a|     8.8±1.1| 12.9±2.3a| 15.9±1.1| 14.6±2.5|
| Diabetics                        |         |           |         |         |         |         |         |
| Group I                          | 10      | 3.2±1.1    | 9.4±1.8a|     7.5±0.8| 13.0±1.1a| 18.5±4.6| 17.9±5.0|
| Group II                         | 7       | 7.2±0.8    | 7.2±1.9a|     8.4±0.9| 13.1±1.7a| 28.0±5.5| 28.8±6.7|
| Group III                        | 8       | 1.5±0.9    | 4.0±1.5a|     8.0±1.0| 11.1±0.9a| 30.5±1.5| 31.5±3.8|
|                                  |         |           |         |         |         |         |         |
| Values given are the mean±S.E.   |         |           |         |         |         |         |         |
| a Significantly different from recumbent condition (p<0.05) within each group.
Group III diabetics were lower than those in the healthy subjects and Group I diabetics (p<0.05, p<0.005, respectively). Serum trypsin levels in Group II diabetics were significantly lower than those in Group I diabetics (p<0.05). As shown in Fig. 1, there was no significant correlation between IRC and serum trypsin (r=-0.30).

Serum $\alpha_2$-globulin levels were $0.56\pm0.04$ g/dl in the healthy subjects, $0.55\pm0.03$ g/dl in Group I, $0.69\pm0.07$ g/dl in Group II and $0.81\pm0.07$ g/dl in Group III diabetics. The values in Group III were significantly higher than those in healthy subjects and in Group I diabetics (p<0.005, respectively). Fig. 2 demonstrated that IRC was significantly correlated with serum $\alpha_2$-globulin (r=0.52, p<0.01). On the other hand, IRC was not significantly correlated with serum albumin, $\alpha_1$, $\beta$ and $\gamma$-globulin levels. The levels of serum $\alpha_1$-antitrypsin and $\alpha_2$-macroglobulin are shown in Fig. 3. Serum $\alpha_1$-antitrypsin levels in the healthy subjects, Group I, II and III diabetics were $228.3\pm9.6$, $304.1\pm13.6$, $268.3\pm6.4$, $278.1\pm16.8$ mg/dl, respectively. Serum $\alpha_1$-antitrypsin was increased in all diabetic groups, as compared with the healthy group, but there was no significant difference among the diabetic groups. The relationship between IRC and serum $\alpha_1$-antitrypsin was not statistically significant (r=0.27). Serum $\alpha_2$-macroglobulin levels in the diabetic groups, $249.7\pm27.0$ mg/dl in Group I, $232.9\pm13.2$ mg/dl in Group II, $299.3\pm35.2$ mg/dl in Group III, were higher than those in the healthy subjects, $151.1\pm9.6$ mg/dl (p<0.05, p<0.01, p<0.01, respectively). Although serum $\alpha_2$-macroglobulin levels in Group III diabetics were higher than those in Group I and II diabetics, there was no significant difference among the three groups. The significant correlation between IRC and serum $\alpha_2$-macroglobulin was observed as shown in Fig. 4 (r=0.61, p<0.01). PRA and PRC were not significantly correlated with serum trypsin, $\alpha_2$-globulin, $\alpha_1$-antitrypsin and $\alpha_2$-macroglobulin, respectively.
Discussion

We reported that low PRA was observed in Group III diabetics with retinopathy and clinical nephropathy, but IRC was significantly higher in Group II and III diabetics than in the healthy subjects (Fujii et al., 1980). A decrease in PRA was detected in the patients with diabetic nephropathy (Christlieb et al., 1976; Perez et al., 1977). Hsueh et al. (1980) suggested that the low PRA in the plasma of those patients could be caused by an impaired conversion from inactive to active renin and high inactive renin levels might be observed. Another possible mechanism related to the elevated IRC in Group II and III diabetics is an increased rate of secretion of inactive renin from the kidney cells. Levels of plasma active renin increase after sodium restriction or upright posture (Atras et al., 1977; Millar et al., 1978). In our study, both PRA and PRC were elevated in all groups after standing, but no significant change was observed in IRC. This result clearly indicates...
that inactive renin was not released under the condition of stress with standing. No significant change in plasma inactive renin was reported after saline infusion in spite of a decrease in active renin (Antonipillai et al., 1981). The stimulation which induces or suppresses active renin release could not be effective enough to release inactive renin from the kidney cells, however, it might provide some effects or some differences among the groups in the time course of standing. It seems that elevated IRC in Group III diabetics was not due to an increased rate of secretion, but rather may be due to an impaired conversion between inactive and active renin.

Recent studies demonstrated that endogenous serine proteases including trypsin and kallikrein were necessary for the activation of inactive renin (Sealey et al., 1978; Funae et al., 1979). Serum trypsin concentration is often subnormal in patients with insulin-dependent diabetes (Dandona et al., 1978). Decreased serum trypsin levels were observed in Group II and III diabetics, but there was no correlation between IRC and serum trypsin. Plasma glycoprotein metabolism was modified in diabetic patients, particularly in those with microvascular complications (Jonsson et al., 1976). Many kinds of glycoproteins are contained in the α2-globulin fraction of the plasma protein. In our study, high serum α2-globulin levels were observed in Group III diabetics and there was a significant correlation between IRC and serum α2-globulin levels. On the other hand, renin substrate is also included in the α2-globulin fraction. Although the plasma renin substrate concentration was not measured in the present study, it has been reported to be low in experimental diabetic rat (Christlieb, 1976). Murakami et al., (1980) reported that insulin promoted the synthesis of renin substrate by the isolated rat liver and suggested that low PRA might be partly due to decreased synthesis of renin substrate. Several different plasma glycoproteins have a pathophysiological importance in inhibiting proteolytic enzymes (Heimberger, 1975). Serum α1-antitrypsin and α2-macroglobulin

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![Graph showing correlation between IRC and serum α2-macroglobulin in diabetics and controls.](image-url)

**Fig. 4.** Correlation between IRC and serum α2-macroglobulin in diabetics and controls.
which are potent protease inhibitors in the plasma, were higher in the diabetic groups than in the healthy group. High levels of serum $\alpha_1$-antitrypsin and $\alpha_2$-macroglobulin have been detected in long-term diabetes or in those with microvascular complications (Jonsson and Wales, 1976). Although IRC was not significantly correlated with serum $\alpha_1$-antitrypsin in our patients, there was a significant correlation between IRC and serum $\alpha_2$-macroglobulin. $\alpha_2$-macroglobulin forms complexes with kallikrein and plasmin as well as other proteases, including trypsin, chymotrypsin, elastase and others (Heimberger, 1975) and it may modulate the activity of these proteases. Herpel (1973) reported that $\alpha_2$-macroglobulin failed to inhibit trypsin after acidification. These data suggest that acidification might destroy protease inhibitors and thus make way for proteolytic activation of inactive renin. Poulsen et al. (1979) found that mouse submaxillary renin treated by guanidine bound the human plasma proteases, $\alpha_2$-macroglobulin, inter-\(\alpha\)-trypsin inhibitor, $\alpha_2$-antithrombin. However, the concentration of serum trypsin and serum protease inhibitors we measured is not necessarily related to the biological activity.

It is possible that there is a possible between increased plasma inactive renin in patients with diabetic microvascular complications and the increased plasma protease inhibitor in those patients. Although our results partly support the idea that high plasma inactive renin levels in those diabetics might result from an impaired conversion between inactive and active renin, further investigation to identify the endogenous activator is necessary.

Acknowledgement

We express our gratitude to Professor K. Yamamoto of The Dept. of Pharmacology and Professor K. Okuda of The Dept. of Laboratory Medicine, Osaka City University Medical School, for pertinent advice, and M. Ohara, Kyushu University, for critical reading of the manuscript.

References


