SHORT COMMUNICATION

Absence of activation in vitro of renin in rat plasma

M. H. DE KEIJZER, A. P. PROVOOST AND F. H. M. DERKX*
Department of Pediatric Surgery, Laboratory for Surgery, and *Department of Internal Medicine I, Erasmus University Medical School, Rotterdam, The Netherlands

(Received 15 September 1981; accepted 12 November 1981)

Summary
1. Rat plasma was subjected at 4°C to various treatments known to convert inactive renin into its active form in human plasma.
2. No statistical differences in plasma renin concentration were found when the levels after the various treatments were compared with that of untreated rat plasma.
3. It is concluded that, in contrast to human plasma, no inactive form of renin is present in rat plasma.

Key words: active renin, inactive renin, plasma renin.

Introduction
Recently it has become clear that, in human plasma, renin (EC 3.4.99.19) circulates mainly in an inactive form. This inactive renin, or prorenin, might be a proenzyme comparable with inactive precursors of other proteolytic enzymes circulating in plasma. Inactive renin in human plasma can be converted in vitro into its active form in various ways [1,2]. These include acid dialysis, cold storage and incubation with proteolytic enzymes.

Rats are widely used to study the role of the renin-angiotensin system in blood pressure regulation and experimental hypertension. Reports on the presence of inactive renin in rat plasma are scarce and controversial. Acid-activatable renin may [3] or may not [4] be present in rat plasma. Here we report on the effect of various treatments in vitro, known to activate inactive renin in human plasma, upon rat plasma renin concentration. No activation of renin was detected in rat plasma in vitro.

Methods
Rats
Adult male rats of a Wistar strain, of about 300 g body weight, were used. The animals were killed by decapitation. Blood was collected from the trunk in test tubes chilled on ice containing EDTA. Plasma was separated by centrifugation in the cold and stored at −20°C until assayed.

Renin determination
Renin concentration was determined by the generation of angiotensin I during a 3 h incubation period with an excess of rat renin substrate at pH 6.5. The incubation medium contained the following angiotensinase inhibitors: disodium EDTA (final concentration 5 mmol/l), phenylmethanesulphonyl fluoride (2.87 mmol/l) and aprotinin (Trasylol, Bayer, 100 k-i.u./ml). At the end of the incubation period the reaction was stopped by heating the test tubes in boiling water. The quantity of angiotensin I formed was measured by radioimmunoassay and expressed as pmol of angiotensin I generated h⁻¹ ml⁻¹ (pmol of ANG I h⁻¹ ml⁻¹).

Activation procedures
Plasma samples of ten individual rats were divided into two parts. One part was stored at 4°C for 24 h; the other part was incubated with trypsin (EC 3.4.21.4). The trypsin was covalently bound to CNBr-activated Sepharose. The final
trypsin concentration was 3000 α-N-benzoyl-L-arginine ethyl ester (BAEE) units/ml and the incubation time 24 h. After removal of the trypsin-Sepharose by centrifugation the treated and untreated plasma were assayed for renin concentration.

In pooled plasma obtained from five rats, various methods of renin activation were tested. Samples of 500 µl of plasma were used. Control plasma was kept at −20°C. Cold activation: plasma was kept at 4°C for 24 h, 48 h or 72 h. Trypsin activation: plasma was incubated with Sepharose-bound trypsin at 4°C, either 3000 BAEE units/ml for 24 h or 72 h, or 6000 BAEE units/ml for 24 h, 48 h or 72 h. Acid activation: plasma was dialysed for 24 h at 4°C against glycine buffer (50 mmol/l), pH 3.3, brought back to pH 7.5 by dialysis and either was kept at 4°C for 24 h or 48 h, or was incubated with plasmin (EC 3.4.21.7; 2 caseinolytic units/ml for 24 h or 48 h). After the completion of the various activation procedures the samples were assayed for the plasma renin concentration in quadruplicate.

Statistics

Data are given as means ± SD. The difference between untreated and trypsin-treated individual plasma samples was tested by using a paired Student’s t-test. Statistical differences between the various activation procedures in pooled plasma were tested by one-way analysis of variance.

Results

Plasma renin concentration of trypsin-treated rat plasma was not significantly different from that of untreated samples. The plasma renin concentration amounted to 17.9 ± 4.0 pmol of ANG I h⁻¹ ml⁻¹ for untreated and 17.2 ± 3.7 pmol of ANG I h⁻¹ ml⁻¹ for trypsin-treated plasma.

The effects of the various activation procedures on pooled rat plasma are given in Table 1. One-way analysis of variance showed that no statistical differences between the various treated samples and untreated plasma were present (F₁₁₂,₃₉ = 1.24). As shown, doubling of the trypsin concentration to 6000 BAEE units/ml also caused no renin activation. This indicates that failure of trypsin to activate renin in rat plasma was not simply due to a low trypsin concentration.

Discussion

The present findings indicate that in rat plasma no form of renin is present that can be activated in vitro. This is in contrast to observations made on human plasma, where only about 20% of the total amount of renin circulates as active renin [5].

Recent reports provide further evidence that plasma renin in the rat may be completely different from that in humans. Acidification of plasma from non-anaesthetized rats resulted in no increase in renin concentration, indicating the absence of acid-activatable renin from rat plasma [4], although others reported a 15% increase upon acid-activation [3]. In rat renal extract high- and low-molecular-weight renins have been found [6, 7]. The high-molecular-weight renin was readily converted into low-molecular-weight renin. Furthermore, in superfusate of renal cortical slices there was no demonstrable activation of released renin by either acidification or kallikrein treatment [8]. Also, renin secreted by isolated perfused kidneys was not activated by exposure to cold, acid dialysis or trypsin [9].

All these findings point to the conclusion that renin is not present in an active form in rat plasma.

Acknowledgment

This study was supported by a grant from the Kidney Foundation of the Netherlands (C 194).

References

Active renin in rat plasma


