PRESENCE OF A RENIN INHIBITOR IN THE PLASMA OF INTACT DOGS

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A method of determining a renin inhibitor in the plasma was developed on the principle that during successive dilution of the plasma the velocity of the renin + substrate reaction falls more slowly in the presence of inhibitor than the degree of dilution, and conversely, the reaction velocity in plasma without inhibitor is reduced more than the degree of dilution of the plasma. This principle is deduced from reaction equations suggested by Dixon and Webb (1966). The presence of renin inhibitor was demonstrated by this method in the plasma of eight of 19 intact dogs studied.

KEY WORDS: renin – angiotensin system; noncompetitive inhibitor of renin; dog plasma.

The level of angiotensin II in the arterial blood is known to be determined by the secretion of renin by the kidneys and its interaction with angiotensinogen. There is indirect evidence that the rate of angiotensinogen breakdown depends not only on the concentration of enzyme and substrate, but also on the presence of inhibitors or activators [1-4]. Poulsen [5], however, denies such a possibility.

The presence of renin inhibitor was studied in the plasma of intact dogs.

EXPERIMENTAL METHOD

A dilution method, by means of which renin inhibitor could be estimated quantitatively without the use of purified preparations of substrate and enzyme, was developed in order to determine the inhibitory effect in native plasma. The method is based on the fact that during successive dilution of plasma the velocity of the renin + substrate reaction should fall more slowly in the presence of inhibitor than the degree of dilution; in plasma without inhibitor the reaction velocity should fall more than would correspond to the degree of dilution. This principle is based on the reaction equations suggested by Dixon and Webb [6]. In the presence of inhibitor the reaction velocity is described by the equation

$$V_0 = \frac{V_{max}}{\left(1 + \frac{K_M}{[S]}\right)\left(1 + \frac{[i]}{K_i}\right)}, \quad (1)$$

where V_0 is the initial reaction velocity; $V_{max} = k_2(E)$ is the maximal velocity when the enzyme is saturated with substrate; K_M is the Michaelis constant; S the substrate concentration; [i] the concentration of inhibitor; K_i the inhibition constant. During n-fold dilution of the plasma, keeping all other conditions the same, the concentrations of enzyme, substrate, and inhibitor fall in accordance with the degree of dilution and the equation assumes the form

$$V_0' = \frac{V_{max}}{n = \left(1 + \frac{K_M \cdot n}{[S]}\right)\left(1 + \frac{[i]}{nK_i}\right)}. \quad (2)$$

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Using a set of different values of n, a system of equations is obtained, and by solving them it is possible to determine the three independent parameters: V_{max}, $K_m/[S]$ and $[i]/K_i$. However, the very low concentration of angiotensin I in dog plasma (0.9 ng/ml in 30 min), the increase in which was used to judge the rate of conversion of angiotensinogen, and also the error of the radioimmune method (10%) used to determine the plasma angiotensin I concentration [7] prevent the required set of plasma dilutions from being obtained with sufficient accuracy. However, even with the aid of a small number of dilutions (by 2 and 4 times) it was possible to demonstrate the presence of inhibitor qualitatively and to determine the degree of inhibition of the renin – substrate reaction in native dog plasma. Turning again to Eqs. (1) and (2) and examining the ratio between the velocities V_0 and V'_0, we obtain

$$\frac{V_0}{V_0'} = \frac{n\left(1+\frac{nK_M}{[S]}\right)\left(1+\frac{[i]}{nK_i}\right)}{\left(1+\frac{K_M}{[S]}\right)\left(1+\frac{[i]}{K_i}\right)}.$$

This ratio becomes smaller than or equal to n if the inequality

$$\frac{nK_M}{[S]} \leqslant \frac{[i]}{K_i} \tag{3}$$

is satisfied; i.e., if a noncompetitive inhibitor is present in considerable concentrations the rate of increase of angiotensin I in the diluted plasma will fall more slowly than the dilution of the plasma. In the absence of a noncompetitive inhibitor, the velocity of the enzyme reaction will be described by the Michaelis–Menten equation:

$$V_0 = \frac{V_{max}}{1+\frac{K_M}{[S]}}, \tag{4}$$

but on dilution of the plasma by n times the reaction velocity is determined by the equation

$$V_0' = \frac{V_{max}}{n\left(1+\frac{nK_M}{[S]}\right)}. \tag{5}$$

The ratio between V_0 and V'_0 will be

$$\frac{V_0}{V_0'} = \frac{n\left(1+\frac{nK_M}{[S]}\right)}{1+\frac{K_M}{[S]}}.$$

It is obvious that with all possible values of $K_M/[S]$, $(V_0/V'_0) > n$, i.e., in the absence of inhibitor, the reaction velocity in the diluted plasma will fall more rapidly than the increase in the degree of dilution of the plasma. The presence of large concentrations of noncompetitive inhibitor can thus be determined qualitatively by examining the ratios between the reaction velocities in whole and diluted plasma.

EXPERIMENTAL RESULTS

The ratio between the rates of increase in the content of angiotensin I in whole plasma and in plasma diluted twice and 4 times by tris-acetate buffer with 0.9% NaCl after incubation for 30 min at 37°C and pH 7.4 was determined for a group of intact dogs. The value of this ratio varied from 1.05 to 3.85. According to the arguments presented above, the plasma of animals in which the value of $V_0/V'_0 > 2$ when diluted twice contains a very small quantity of inhibitor, whereas plasma from animals with ratio $V_0/V'_0 < 2$ evidently contains noncompetitive inhibitor in considerable concentrations. According to this criterion the animals were divided into two groups: group 1 consisted of animals with a value of V_0/V'_0 from 3.85 to 2.15, and group 2 animals with a value of V_0/V'_0 from 1.05 to 1.9 (the mean values are given in Table 1). For the animals of group 1, using Eqs. (4) and (5), after transformation we obtain

$$\frac{K_M}{[S]} = \frac{n-\frac{V_0}{V_0'}}{\frac{V_0}{V_0'}-n^2}.$$

TABLE 1. Distribution of Dogs by Concentration of Renin Inhibitor in Plasma (M±m)

Group of animals	No. of animals	Activity of renin in whole plasma, V_0 (in mg/ml/30 min)	Ratio between rate of increase of angiotensin in whole plasma and rate in plasma diluted twice, V_0/V'_0	Ratio between rate of increase of angiotensin in whole plasma and rate in plasma diluted 4 times		Deviation of calculated value from experimental (in %)
				v_0/v''_0	calculated value	
1	11	0,93±0,20	2,78±0,17	8,35±1,23	8,65	3
2	8	1,08±0,30	1,54±0,11	3,45±0,62	2,90	15

Substituting in this equation the mean value of the ratio V_0/V'_0 calculated for this group with n = 2 (Table 1), we obtain the mean value of $K_M/[S]$. It is 0.64. According to data in the literature, the substrate concentration in dog plasma is 776 ng/ml, and K_M = 670 ng/ml [8], i.e., the value of $K_M/[S]$ obtained by the present method was of the same order. For the animals of group 2, from Eqs. (1) and (2), we obtain

$$\frac{[i]}{K_i} = \frac{\frac{V_0}{V'_0}\left(\frac{\frac{K_M}{[S]}+1}{\frac{K_M}{[S]}n+1}\right)^{-n}}{1-\frac{V_0}{V'_0}\left(\frac{\frac{K_M}{[S]}+1}{\frac{K_M}{[S]}n+1}\right)}. \quad (6)$$

Considering that the mean value of $K_M/[S]$ was the same for both groups of animals, and substituting in Eq. (6) the mean value of V_0/V'_0 determined for this group at n = 2 (Table 1), the degree of inhibition of the reaction can be calculated – $[i]/K_i$. In the present experiments, $[i]/K_i$ = 8.25, i.e., in these animals the reaction between renin and substrate was inhibited on the average by 9 times.

Returning to the inequality (3) and substituting in it the value found for $K_M/[S]$ for n = 2, it will be seen that the ratio $V_0/V'_0 \leq 2$ is satisfied under the condition that $[i]/K_i \geq 1.28$; i.e., under the conditions of the present experiments, if the plasma was diluted twice, only degrees of inhibition by more than 2.3 times could be estimated. By reducing the degree of dilution the sensitivity of this method could be increased. To verify the validity of these arguments the following method was used. Knowing $K_M/[S]$ and $[i]/K_i$, the ratio between the rates of increase of angiotensin I in undiluted plasma and in plasma diluted fourfold (V''_0) was calculated for the animals of groups 1 and 2 and compared with the values obtained experimentally. Good agreement was obtained: The deviation between the values in group 1 was 3% and in group 2 2.15%, so that the validity of the original hypothesis regarding the presence of inhibitor in the native plasma of the animals of group 2 was confirmed.

The presence of noncompetitive inhibitor of the renin + substrate reaction in the plasma of intact dogs was thus demonstrated by the suggested method. The results showed that its concentration may vary within wide limits in different animals. Hence it is clear that, despite the virtually equal activity of renin in the plasma of both groups of dogs (Table 1), the concentration of the enzyme is significantly less in the animals of group 1 than in those of group 2, in which a renin inhibitor is present. Consequently, the renin–angiotensin system may be controlled not only by changes in the secretion of renin by the kidneys, but also by the intervention of unknown factors which alter the concentration of renin inhibitor in the plasma.

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