TISSUE KALLIKREIN ASSAY IN BLOOD PLASMA AND ITS CLINICAL IMPORTANCE

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Experimental research in the last 3 or 4 years, using methods of immunoradiology and preparative biochemistry, has shown that endogenous plasma kallikrein and kallikreins of tissue origin can circulate simultaneously in the blood stream of man and animals [11-15]. Tissue kallikreins include kallikreins of the kidneys [3, 4, 16], pancreas [17], and salivary [5, 8, 10, 15] and sex glands [6]. Most of the tissue kallikrein in the blood is considered to be from the salivary glands [8, 11, 15]. It has also been shown that in pathology (acute pancreatitis or nephrectomy) the blood level of tissue (pancreatic or renal) kallikrein rises. For instance, a 2.5-fold rise in the serum renal kallikrein level has been observed [16] in patients with acute pancreatitis (9.5 ± 1.7 ng/ml compared with the normal value of 3.8 ± 0.7 ng/ml) and a 4.5-fold rise [7] in the blood plasma of rats after bilateral nephrectomy (162 ± 20 and 618 ± 79 ng of antigen in 1 ml plasma respectively).

The results are evidence of the importance of combined and separate quantitative assay of the kininogenase activity of the plasma kallikrein and kallikrein of tissue origin during a study of the physiological and pathogenetic role of kinins and the development of methods of experimental therapy, aimed at correcting pathologically changed kininogenesis.

There is as yet no sufficiently simple clinical method of determining kallikreins derived from glands in human plasma or blood serum. A complex immunoradiometric method has been described (using antisera from rabbits, sheep, and goats against purified preparations of kallikrein from rat urine and salivary glands), of determining kallikrein derived from urine [7, 16] and salivary glands [5] in rat plasma [5, 7] and in human serum [6].

The aim of this investigation was to develop an accessible method whereby kallikrein of tissue origin can be determined in blood plasma from healthy subjects and patients.

EXPERIMENTAL METHOD

Development of the method was based on the well-known fact that soy trypsin inhibitor (STI) has qualitatively different effects on plasma kallikrein and tissue kallikrein. This inhibitor inhibits plasma kallikrein and its complex with high-molecular-weight kininogen [9], but does not affect kallikreins derived from glands [3].

In experiments in vitro with blood plasma from healthy blood donors concentrations of STI causing 30-50% inhibition of total kallikrein activity in blood plasma, estimated by the kininogenase method [2] with determination of the quantity of kinins in the isolated uterine cornu of Wistar rats, were selected. Quantitative estimation of tissue kallikrein in blood plasma was based on determination of total plasma kallikrein activity [2], the degree of inhibition of this activity in the presence of STI, and calculation of the concentration of STI inducing inhibition of kininogenase activity of kallikrein by 50%.

The STI and some of the bradykinin triacetate used in the work were from Reanal (Hungary); other bradykinin triacetate was of Soviet manufacture, and the kaolin and Tris also were of Soviet origin.

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TABLE 1. Effect of STI on Kininogenase Activity of Plasma Kallikrein (inhibition, in %) and on Activity of Tissue Kallikrein (in pmoles/sec · liter) in Inflammatory and Endocrine Diseases (M ± m)

Groups of subjects tested	Number of subjects	Dose of STI, μg/ml			Total plasma kallikrein	Tissue kallikrein
		0,5	1	10		
Healthy persons	10	31	60	90	391,14±39,36	147,6±12,3
Patients with Active form of diabetes	40	2	10	44	548,58±44,28	3072,05±22,62
Diabetes with disturbed glucose tolerance	13	9	21	54	912,66±66,42	4198,20±34,57
Secondary diabetes	4	0	0	48	519,06±36,90	2699,11±24,6
Cushings disease	8	—	60	93	393,60±36,90	157,44±13,73
Acute suppurative inflammation	9	30	66	88	595,32±51,66	202,41±26,28
Chronic pneumonias	11	33	64	90	221,40±19,68	72,28±4,17
Acute pancreatitis	8	30	60	86	221,40±29,52	88,56±7,38
Bronchitis due to dust	24	17	36	68	620,32±30,54	396,80±19,10
Chronic hepatitis	9	20	40	76	629,76±39,62	378,84±18,51

Legend. Here, and in Tables 2 and 3, activity of all forms of kallikrein is expressed in picomoles bradykinin hydrolized per second by 1 liter of blood plasma.

TABLE 2. Inhibition of (in%) Kininogenase Activity of Plasma Kallikrein by STI Depending on Prekallikrein Content in Patients with Diabetes (M ± m)

Group of subjects tested	Number of subjects	Prekallikrein content, picomoles/sec · liter	Dose of STI, μg/ml		
			0,5	1	10
Patients with diabetes	10	157,44±17,22	31	60	90
Active form (group I)	34	4,42±0,49	0	6	37
Active form (group II)	16	98,40±9,84	8	21	58
Active form (group III) with disturbed glucose tolerance	9	88,56±9,84	9	21	54

TABLE 3. Changes in Tissue Kallikrein by STI in Patients with Diabetes before and after Treatment (M ± m)

Type of treatment	Number of subjects	Time of investigation	Dose of STI, μg/ml			Kallikrein, picomoles/sec · liter	
			0,5	1	10	Plasma	Tissue
Heparin (microdoses)	8	Before treatment	0	0	37	182,04±19,68	1173,42±100,86
		After treatment	14	50	92	378,84±51,66	189,42±14,76
Andekalin (ordinary doses)	9	Before treatment	0	13	47	258,30±22,14	1377,60±152,52
		After treatment	0	21	51	376,38±56,74	1820,40±174,66

EXPERIMENTAL RESULTS

The series of experiments with blood plasma from 20 blood donors showed that incubation of neutralized prekallikrein-kallikrein-kininogen (PK-K-KG) mixture, obtained after heating the test blood plasma in an acid medium (pH 3.0) at 61°C for 17-18 min with STI in a concentration of 0.5 μg/ml of mixture led to inhibition of kininogenase activity by 30% compared with its level in a parallel test sample to which STI was not added. An increase in the STI concentration in the PK-K-KG mixture by 2-20 times led to inhibition of the original kininogenase activity of the total kallikrein of the above mixture by 60-90%.

Investigation of plasma from patients, depending on the diagnosis of the disease, showed that high doses of STI (1-10 μg/ml) were needed to inhibit the original kininogenase activity of total plasma kallikrein by 30-50%.

Trials of the various methods suggest the following course of the biochemical reaction for simultaneous determination of activity of tissue (without specifying the concrete organ) and total (including tissue) blood plasma kallikreins.

To 1.2 ml of plasma are added 1.2 ml of 0.14 M NaCl and 0.3 ml of 1N HCl solution, pH 3.0; the mixture is heated for 17-18 min to 61°C, cooled to 2-4°C, and neutralized with cold 0.5N NaOH solution (0.54 ml), and 3.3 ml of Tris-HCl, pH 7.6-7.8, at the same temperature is added. In this way the PK-K-KG mixture is obtained, in a total volume of 6.6 ml. Into each of six empty test tubes, numbered serially, the following ingredients are added: 0.2 ml of 0.14 M NaCl (sample No. 1 without kaolin), 0.1 ml of Tris-HCl containing 0.5 and 5 mg kaolin (samples Nos. 2 and 3), 0.5, 1, and 10 μg of STI (samples Nos. 4-6). Additionally 0.1 ml of 0.14 M NaCl is added to each of the tubes Nos. 2 and 3, and 0.5 mg kaolin in 0.1 ml of physiological saline is added to each of the tubes Nos. 4-6. Into all tubes (Nos. 1-6) an equal volume (1.1 ml) of the cold PK-K-KG mixture is added, allowed to stand for 5-10 min at room temperature, and the tubes are then incubated for 1 h at 37°C. The reaction is stopped by the addition of 0.5 ml of 10% TCA, proteins are separated by centrifugation, and the kinin content is determined in neutralized TCA supernatants on the cornu of the rat uterus. Synthetic bradykinin is used as the standard.

By determining kininogenase activity of samples Nos. 1-3 the characteristics of three forms of kallikrein can be obtained: in complex form with inhibitors, and including kallikrein of tissue origin [5] (sample No. 1 without kaolin), total kallikrein (sample No. 2 with 0.5 ml of kaolin), and prekallikrein (difference between activities of samples Nos. 2 and 1), and also an index of absorption of kallikrein on kaolin in per cent (the difference between activities of samples Nos. 2 and 3), reflecting changes in the conformational structure of the kallikrein [2]. Taking activity of sample No. 2, not containing STI, but reflecting total plasma kallikrein activity and acting as control for samples Nos. 4-6, as 100%, the percentage of inhibition of their kininogenase activity is found. Activity of all forms of plasma and tissue kallikrein is expressed in picomoles of bradykinin hydrolyzed per second by 1 liter of blood plasma. Tissue kallikrein activity is estimated quantitatively allowing for an STI concentration of 1 μg/ml, causing 30-50% inhibition of the kininogenase activity of the total blood plasma kallikrein. With the dose of STI used, corresponding to 10 μg to 1 ml of mixture, activity of the samples is increased tenfold. By the method described above, kallikrein of tissue origin was determined in the blood plasma of 10 clinically healthy persons and of 136 patients with various inflammatory and endocrine diseases.

Analysis of the data in Tabel 1 indicates a 10-20-fold increase in the concentration of kallikrein of tissue (probably pancreatic) origin, differing in its biochemical properties from blood kallikrein [1], in the blood stream of patients with diabetes (both with the active form and with the form with disturbed glucose tolerance), and a 60% increaee in tissue kallikrein activity was found in patients with acute abscesses, carbuncles, and furunculosis. A 2.5-3-fold increase in the tissue kallikrein concentration was found in the plasma of patients with chronic bronchitis due to dust (50%) and with chronic hepatitis (30%).

In patients with diabetes direct correlation was found between the concentration of prekallikrein (which is synthesized mainly by the liver) and the degree of inhibition of total plasma kallikrein activity by STI (Table 2). As will be clear from Table 2, the lower the level of the precursor of the kinin-forming enzyme in the plasma, the more STI is required to inhibit kininogenase activity of total blood kallikrein.

It was also shown that correction of depressed kininogenesis (which was found in 30% of patients with diabetes) by means of a course of injections of microdoses of heparin led to normalization of the blood kallikrein composition. Treatment of such patients with the usual doses of andekalin (10-20 U/day for 2-3 weeks) did not change the kallikrein composition of the blood (Table 3).

This combined method of simultaneous determination of the various forms of blood plasma kallikrein and kallikrein of tissue origin present in the plasma can thus yield information of use in the study of the physiological role of tissue kallikreins in the regulation of vascular tone; it can shed light on the pathogenetic role of the kallikrein-kinin system in the development of various diseases, can be used to determine the state of the liver function in diseases of the liver itself and in other pathological states, and can be used as a method of laboratory control of the therapeutic efficacy of a chosen method of treatment.

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