COMPARISON OF BIOLOGICAL ACTIVITY OF ISCHEMIC TOXIN AND ITS EFFECT ON CHEMILUMINESCENCE OF BLOOD PLASMA

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UDC 616-005.4-07:616.15-008.6-074

KEY WORDS: chemiluminescence of blood plasma; ischemic toxin; biological activity.

Previous investigations showed that during hemorrhagic shock and ischemia of individual organs (liver, small intestine) the intensity of chemiluminescence (CL) of blood plasma induced by Fe^{++} ions, due to the appearance of compounds of average molecular weight in the blood stream [2, 6], is depressed. On the other hand, substances with a molecular weight of about 10,000 daltons, with a marked toxic action and known as ischemic toxin (IT), appear in the blood stream during ischemia [3-5, 8]. Since both factors, the quencher of CL and the toxin, appear under the same conditions (ischemia), and since they have a similar molecular weight, of the order of 10,000 daltons, the question arises whether IT may in fact be a substance with the property of quenching CL. If so, the chemiluminescence method could be used for the rapid detection and assay of IT in the blood.

EXPERIMENTAL METHOD

A mixture of polypeptides was isolated from blood plasma, obtained after ischemization for 6 h resulting from application of a tourniquet to a dog's limb, by differential ultrafiltration followed by fractionation on Sephadex G-15 [3, 4]. The elution curve recorded by measuring optical density of the solution flowing from the column at 280 nm, had several peaks; the fractions corresponding to them were collected and their biological activity and CL was measured. Most of the toxic material was eluted within a volume of 250-280 ml (fractions IIa and III), the deadspace volume of the column being 200 ml [4, 5]. It was these fractions which together constituted the "ischemic toxin."

Biological activity of fractions I-VII was determined on the isolated frog's heart and on the frog's heart *in situ*, by injection of the test preparations in a volume of 0.1 ml into the right atrium through a catheter introduced into a vein. The amplitude of the cardiac contractions was recorded by pneumatic transmission on a Mingograph 81. The maximal amplitude of contractions was established practically at once after injection of the test doses of the preparation. The increase in amplitude was expressed as a percentage of the amplitude after injection of Ringer's solution. This increase in amplitude was designated PA.

To measure Cl, 0.5 ml blood plasma from an intact dog, 1.0 ml of a solution of one of the IT fractions in concentrations (as protein) of between 10 and 70-100 μg/ml, and 7.5 ml phosphate buffer mixture (105 mM KCl and 20 mM KH_2PO_4) were introduced into the constant-temperature cuvette (37°C) of a CL recording apparatus [1, 6]. After addition of 1.0 ml of a 10 mM solution of $FeSO_4 \cdot 7H_2O$ the development of CL was recorded [1, 2, 6].

EXPERIMENTAL RESULTS

It will be clear from Fig. 1A that in the presence of IT the amplitude of the "slow flash" of CL (I_{max}) was reduced and the time of development of the flash τ was increased. Since a definite relationship was found between these two characteristics (Fig. 1C), and since it was the same with all fractions used, to describe the quenching action of the toxin it was decided in future to use only the value of I_{max}/I_o, where I_o is the amplitude of the "slow flash" of CL recorded in the absence of toxin.

Department of Biophysics, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 20-22, July, 1981. Original article submitted January 16, 1981.

 0007-4888/81/9207-0876$07.50

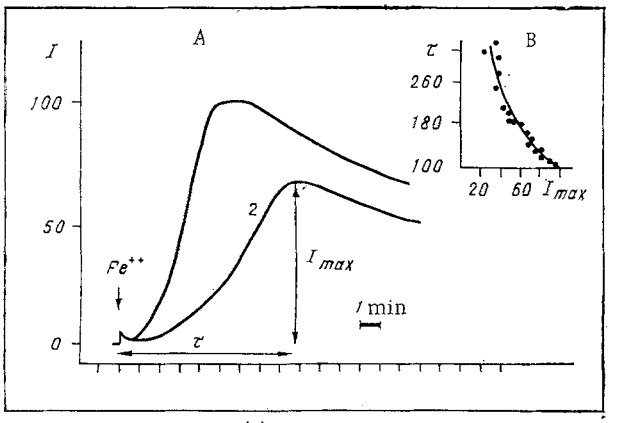

Fig. 1. Effect of IT on Fe^{++}-induced CL of blood plasma. A) Curve of "flash" of CL: 1) blood plasma from intact dog; 2) with addition of 5.6 μg/ml fraction III to blood plasma. Abscissa, time (in min); ordinate, intensity of CL (in relative units); B) correlation between I_{max} and τ.

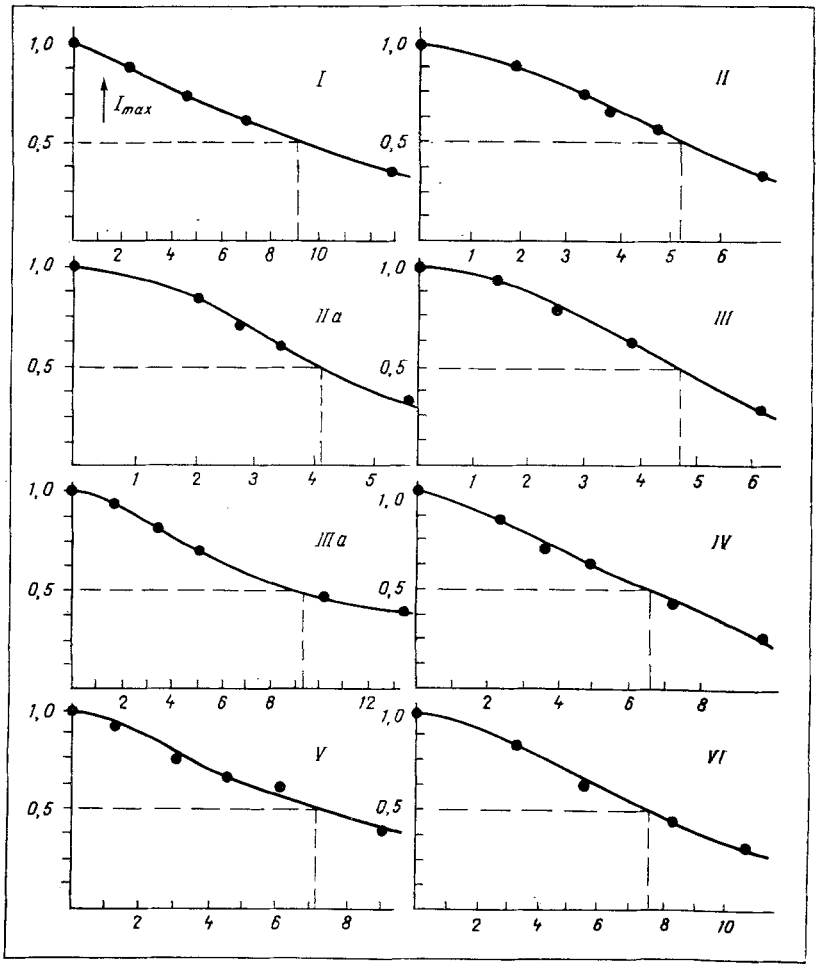

Fig. 2. Effect of various low-molecular-weight fractions of blood plasma on intensity of its CL. Abscissa, concentration of test fractions (in μg/ml); ordinate, maximal amplitude of CL (in relative units). Roman numerals denote fraction Nos.

Curves showing the relationship between dose of the various fractions and amplitude of I_{max} are given in Fig. 2. The mechanism of action of the various fractions on CL is clearly similar, and they differ only in their activity. As formal index of activity, the reciprocal of the concentration inducing a certain degree of quenching of CL, by half, for example,

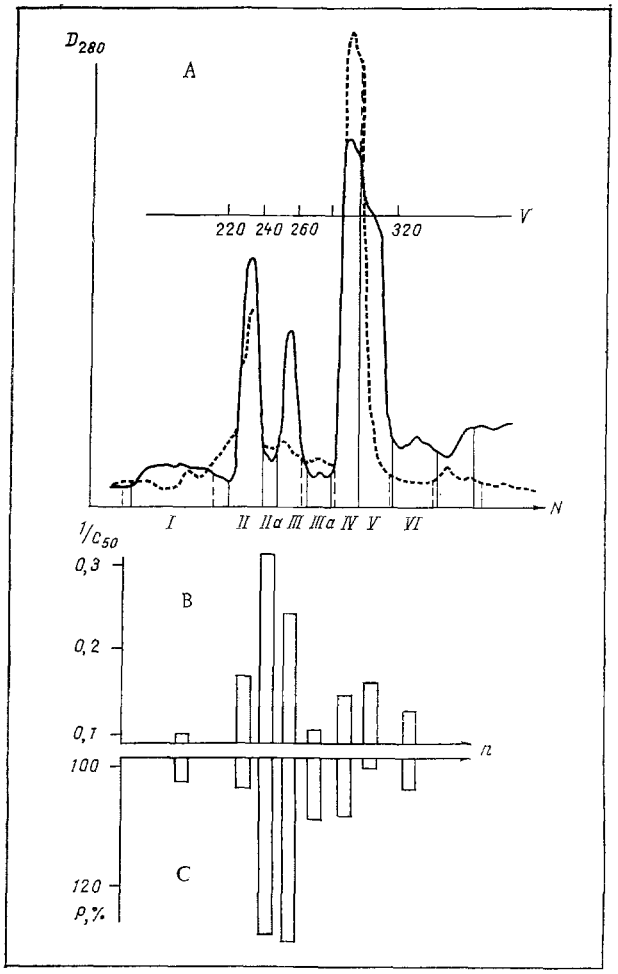

Fig. 3. Characteristic elution curve of dog blood plasma purified beforehand by differential ultrafiltration (A) and physicochemical (B) and biological (C) activities corresponding to isolated fractions. n (I, II, . . ., VI) Nos. of isolated fractions; V) elution volume (in ml); D) optical density at 280 nm. Broken line represents elution curve before application of tourniquet; continuous line — curve after ischemization for 6 h.

as was done in [7], can probably be used. Activities of the different fractions, expressed thus ($1/C_{50}$), are compared in Fig. 3 with the biological activity of the same fractions, expressed in terms of the index P of their action on cardiac contractions. Fractions eluting in the 250-280 ml volume, designated II, IIa, and III, and generally described as "ischemic toxin," were found to be most active. These fractions, in a dose of 6.0 μg protein, gave rise to a marked biphasic reaction, consisting in initial stimulation and subsequent depression of amplitude of the cardiac contractions without any significant change in their rhythm. These same fractions, biologically most active, possessed at the same time the strongest ability to quench CL. The low-molecular-weight fractions V, VI, and VII, and also fraction I, which had no biological activity, had weak ability to quench CL.

The results are evidence that fractions II, IIa, and III, which contain most of the IT, quench CL. The mechanism of the quenching effect is not yet clear. However, it is evidence that the phenomenon of quenching of CL can provide a sound basis for the subsequent development of a method of determining IT in the blood of affected animals and also, possibly, of human patients with various forms of disease.

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ROLE OF SEROTONIN IN THE PATHOGENESIS OF ULCER FORMATION

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UDC 616.33-002.44-092:577.175.823

KEY WORDS: ulcer formation; serotonin; proteolytic enzymes; lysosomal enzymes.

The largest quantity of serotonin in the body is produced in the stomach. However, the question whether this biogenic amine plays the role of a trigger mechanism for gastric secretion or whether its liberation is the result of the initiated process of digestion has not been completely elucidated. Meanwhile it is evident that a disturbance of serotonin synthesis and metabolism can lead to the development of pathological states of the gastrointestinal tract.

The results of investigations to determine serotonin in patients with peptic ulcer have proved fairly contradictory. According to some workers correlation is found between the acid-forming function of the stomach and the serotonin concentration in the gastric juice of such patients [2, 3]. According to other workers [14] serotonin regulates equilibrium between HCl and pepsin and also regulates the mucosal barrier and cell regeneration. There have been few investigations to determine serotonin in the gastric mucosa. In peptic ulcer an increase in the serotonin concentration has been found in the mucous membrane of the stomach and duodenum [6]. However, much remains to be explained regarding the role of serotonin in the genesis of ulcer formation.

The object of this investigation was to study the serotonin concentration in the mucous membrane of the rat stomach at various stages of experimental ulcer formation. At the same time the concentration of histamine and the proteolytic activity and spectrum of proteolytic enzymes were determined in the mucous membrane. Changes in the acid protease level in the gastric mucosa after administration of serotonin and histamine also were determined in intact animals.

EXPERIMENTAL METHOD

Experiments were carried out on 150 albino rats of both sexes, kept on the ordinary laboratory animal diet. The animals were deprived of food for 16-18 h before the investigations but were allowed water *ad lib*. The animals were divided into three groups: 1) intact, 2) control and undergoing laparotomy with application of physiological saline, and 3) experimental rats with application of acetic acid to the serous membrane of the anterior wall of the body of the stomach [12]. Some animals of group 1 were given a known ulcerogenic dose of serotonin (30 mg/kg, intraperitoneally) or histamine (50 mg/kg, intramuscularly). Pieces of mucosa for testing were taken from the floor and margin of the ulcer, and from the corresponding part of the stomach of the control rats.

Proteolytic activity was determined in extracts of gastric mucosa by the method in [8] and the pH-curve of proteolytic activity was plotted [1]. The concentrations of serotonin

Laboratory of Pathophysiology, Central Research Institute of Gastroenterology. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 23-36, July, 1981. Original article submitted June 11, 1980.

0007-4888/81/9207-0879$07.50