ANAPHYLACTOGENIC ACTIVITY OF PROTEINASES FOR MEDICAL USE

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The anaphylactogenic activity of proteinases for medical use (terrilytin, hygrolytin, trypsin, and chymotrypsin) was studied in active anaphylaxis experiments on guinea pigs. Differences in the allergizing potential of the enzymes were established by the use of the anaphylactic index and minimal sensitizing dose. Enzymes of microbial origin had stronger sensitizing properties. No correlation was found between the strength of the anaphylactic reaction and the intensity of the humoral immune response.

KEY WORDS: anaphylaxis; proteinase.

Enzymes have now secured a firm place in the arsenal of chemotherapeutic agents. In the USSR and elsewhere medicinal forms of microbial enzymes are marketed for use in the treatment of diseases of the respiratory and digestive organs and in various other pathological states [7, 13]. Preparations intended for the treatment of thromboembolic diseases, including urokinase, streptokinase, and brinase [10], are at the stage of development and introduction. Besides their undoubted therapeutic value, experience of the use of thrombolytic and other enzymes have brought to light evidence of their ability to cause sensitization of sensitive individuals [9, 10]. Accordingly the development of criteria characterizing the allergizing potential of enzymes for medical use is of the utmost importance.

The object of this investigation was to study the anaphylactogenic activity of proteolytic enzymes of Soviet manufacture for medical use and to study correlation between the results and those of investigation of the humoral immune response.

EXPERIMENTAL METHODS

Proteinases of microbial origin, namely terrilytin and its basic component, proteinase I, hygrolytin (preparations 4G and 200/10) obtained at VNITIAF, and also trypsin and chymotrypsin (manufactured by the Leningrad Meat Combine), were used.

The anaphylactogenic properties of the enzymes were studied on guinea pigs weighing 300-350 g. The animals were sensitized by single intracardiac injections of the preparations in doses of $5 \cdot 10^{-1}$ to $5 \cdot 10^{-6}$ mg protein/kg body weight. The reacting injection was given 3 weeks later into the popliteal vein. The enzyme for the reacting injection was used in a concentration which, when injected into intact animals, gave rise to no toxic reactions: It was usually 5 to 10 times greater than the sensitizing dose. At least five animals were used for each dose of each preparation. The intensity of the anaphylactic reaction was assessed by a four-point scale [3]. Experimental data were analyzed by the method of Weigle et al. [15] and the anaphylactic index (AI) was calculated by the following equation:

$$AI = \frac{(a \cdot 4) + (b \cdot 3) + (c \cdot 2) + (d \cdot 1) + (e \cdot 0)}{a + b + c + d + e},$$

where a is the number of animals which died (degree of shock 4+), b the number of animals with severe shock (3+), c the number of animals with a moderate reaction (2+), d the number of animals with mild shock (1+), and e the number of animals not reacting to the second injection of anitgen.

To study the humoral immune response to the enzyme the indirect hemagglutination test was used (IHT). Blood was taken by cardiac puncture 1-2 days before injection of the reacting doses of the antigen. Enzyme–formalinized sheep's red blood cell conjugates were used as test antigens for the IHT [11, 14].

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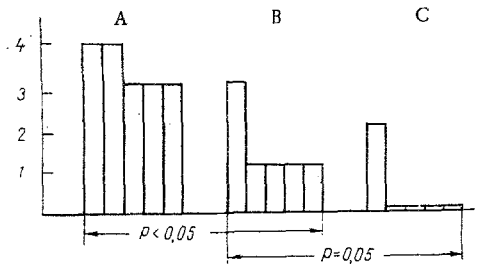

Fig. 1. Intensity of anaphylactic shock in guinea pigs sensitized with terrilytin, depending on dose of reacting injection. A) 1.6 mg protein/kg, B) 1.0 mg protein/kg, C) 0.25 mg protein/kg body weight. Ordinate, intensity of shock (in points).

TABLE 1. Anaphylactogenic Properties of Enzymes

Enzyme	AI for sensitization with undermentioned doses, mg protein/kg					
	5×10^{-1}	5×10^{-2}	5×10^{-3}	5×10^{-4}	5×10^{-5}	5×10^{-6}
Terrilytin	1,8	1,0	1,3	0,3	0,4	0,8
Proteinase I	*	0,8	1,0	0,2	0	0
Hygrolytin 200/10	3,0	1,3	0	0,3	0,4	0
Hygrolytin 4G	2,3	1,4	2,0	2,0	2,5	1,3
Trypsin	2,0	0,8	0,6	0,5	0	0,5
Chymotrypsin	0,8	1,0	1,0	0,6	1,0	0

Legend. *) In this concentration the enzyme caused toxic death of the animals. 0) No anaphylactic reaction present.

EXPERIMENTAL RESULTS

The strength of an anaphylactic reaction is largely dependent on the mode of injection of the antigen and the size of the dose used for the reacting injection [1-3]. To obtain a clear picture of anaphylactic shock, the dose of antigen given in the reacting injection must be several times greater than the sensitizing dose. Preliminary experiments showed that intracardiac injection of comparatively small doses of proteolytic enzymes is impossible, for it gives rise to a marked pseudoanaphylactic reaction in intact animals terminating in most cases in death. The cause of this phenomenon is evidently proteolysis of proteins of heart muscle and other sensitive tissues. Accordingly the method of injecting the enzyme into the popliteal vein [3] was used, for in this way the dose of proteolytic enzymes tolerated by intact guinea pigs could be considerably increased. For terrilytin, for instance, this dose could be increased eightfold. The importance of selection of the optimal dose for the reacting injection is shown by the results of experiments with animals sensitized with terrilytin (Fig. 1).

Having determined the dosage for the reacting injections it was possible to move on to the next stage of the work, the aim of which was to determine the anaphylactogenic properties of all the proteinases used. The results of these experiments are given in Table 1.

As these results show, the allergizing potential of the proteolytic enzymes varied within wide limits. In most cases, however, the strength of the anaphylactic reaction for the sensitizing dose of proteinases of animal origin tested was lower than for microbial enzymes. Similar relationships also were observed during a study of the humoral immune response to these enzymes in experimental animals [4].

Differences in the anaphylactogenic activity of the hygrolysin preparations 200/10 and 4G, obtained from different strains of Actinomyces hygroscopicus, will be noted. It is very likely that protein impurities, or perhaps differences in their content, are responsible for the different levels of allergizing potential. The fact that protein impurities can in fact increase the sensitizing activity of the basic enzymes is supported by data in the literature [12], and also by comparison of the anaphylactogenic activity of terrilytin and of its basic component – immunologically pure proteinase I. The results now obtained show that to reduce their allergizing potential, microbial proteolytic enzymes intended for parenteral administration must be purified to the greatest possible degree.

TABLE 2. Humoral Immune Response in Sensitized Guinea Pigs

Antigen	Geometric mean antibody titer ($\log_2$) in sera of animals						
	intact	immunized with enzymes in the following doses, mg protein/kg					
		5×10^{-1}	5×10^{-2}	5×10^{-3}	5×10^{-4}	5×10^{-5}	5×10^{-6}
Proteinase I	2,8	—	3,7	3,5	3,5	3,0	3,0
Hygrolytin 200/10	2,5	—	5,6	3,5	4,0	4,7	2,8
Hygrolytin 4G	2,5	4,8	4,2	3,5	2,4	4,7	2,7
Trypsin	2,0	1,6	1,5	2,3	1,5	1,2	1,5
Chymotrypsin	1,3	2,2	2,0	3,5	3,2	2,6	—

The most marked anaphylactic reactions were observed after sensitization with the maximal dose of $5 \cdot 10^{-1}$ mg protein/kg. With a decrease in the dose taken for sensitization, the strength of anaphylactic shock was weakened and there was a corresponding decrease in the value of AI. Judging from the results, a value of AI of between 1.0 and 1.3 must be considered as the threshold. Clearly the lower the allergenic activity of an enzyme, the greater the minimal sensitizing dose must be, and the lower will be the values of AI. AI and the minimal sensitizing dose are thus important criteria of the allergizing potential of enzymes.

On the basis of the experimental results the various enzymes can be arranged in the following order of decreasing anaphylactogenic activity: hygrolytin 4G > terrilytin > hygrolytin 200/10 > proteinase II-I > trypsin > chymotrypsin.

Since it is generally accepted [1, 2, 8] that anaphylactic reactions are due to antibodies, the next step was to determine the character of the humoral immune response to the enzymes studied. The IHT which, according to many authorities [3, 6], is a highly sensitive method of detecting various antibodies, was used for this purpose.

By contrast with the previous experiments, it cannot be concluded from the results of determination of antibodies in the blood sera of the immunized animals that there is any clear relationship between the intensity of the humoral immune response and the scale of the sensitizing dose (Table 2). However, it cannot fail to be noticed that the titers of the sera of the sensitized guinea pigs were higher in most cases than those of normal sera. These differences were observed both in the group of animals responding with shock to the second injection of enzymes and also in the group of animals which virtually did not respond to the reacting injection of antigen. However, in all cases in animals with a marked picture of shock (3+ to 4+) antibodies were found. The positive results of the IHT could therefore indicate the potential possibility of obtaining systemic anaphylaxis in response to injection of adequate doses of antigen.

Synthesis of the antibodies detectable by the IHT took place more actively in response to injection of microbial enzymes than to injection of proteinases from animal tissues. In the preparations hygrolytin 200/10 and 4G, which differed significantly in their anaphylactogenic properties, the immune response was about the same over the whole dose range of enzymes used for immunization.

The absence of any direct correlation between the strength of the anaphylactic reaction and the intensity of the humoral immune response can evidently be explained on the grounds that antibodies causing anaphylactic reactions in guinea pigs and antibodies detectable by the IHT belong to different classes of immunoglobulins, the production of which in vivo, as data in the literature indicate [5, 8], is competitive in character.

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