SERUM KININOGEN CONCENTRATION IN RABBITS WITH STREPTOCOCCAL ALLERGY OF IMMEDIATE TYPE

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In the course of sensitization with cytoplasmic antigens of group A streptococci the serum kininogen concentration in rabbits is increased. In the early stages and at the height of sensitization, elevation of the kininogen level coincides with an increase in the titer of precipitins and in the percentage of allergic modification of the leukocytes. In the late stages of sensitization no such coincidence is observed. In anaphylactic shock the kininogen concentration falls sharply while the titer of precipitins is substantially unchanged.

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Among the numerous humoral factors (histamine, acetylcholine, serotonin, etc.), whose participation in the pathogenesis of allergic processes has been demonstrated, special attention in recent years has been paid to the vasoactive polypeptides (kinins), which, together with the enzymes forming them, constitute the kinin system. A particularly important member of the group is bradykinin, which is formed from an inactive precursor or kininogen (bradykininogen), located in most mammals in the α_2-globulin fraction of the blood serum [13]. In rabbits, however, the kininogen is an α_1-glycoprotein [1].

The kininogen level is modified under physiological and pathological conditions [4, 14-16], and nowadays the participation of this powerful vasodilator substance in the pathogenesis of anaphylaxis can be considered as proved [6-12].

So far, however, the dynamics of the concentration of kinins in the course of sensitization, and also during allergy of the immediate type produced by bacterial antigens, has not been studied. The study of this problem is not only of theoretical, but also of practical interest since bacterial allergy is a dominant factor in the pathogenesis of several diseases.

In the present investigation the serum kininogen concentration was studied in the course of streptococcal sensitization and in anaphylactic shock.

EXPERIMENTAL METHOD

Experiments were carried out on 26 male rabbits weighing 3 kg. The animals were sensitized with cytoplasmic antigens of group A streptococci obtained by Tustanovskii's method [5]. The protein content of the solutions was 3 mg/ml. Sensitization was carried out by alternate subcutaneous injections of antigen into the right and left inguinal regions in a volume of 0.5 ml daily for five days. The degree of sensitization was judged from the antibody titer and the number of destroyed leukocytes after exposure to the specific antigen.

The antibody titer was determined by the ring precipitation test. To perform this test the antiserum was diluted 1 : 2. The results of the test were read during a period of 1 h.

To study modification of the leukocytes the method of luminescence microscopy of leukocytes fluorochromed intravitally with acridine orange [2] was used.

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TABLE 1. Kininogen Contraction (in μg bradykinin/ml) in Serum of Rabbits and Blood Pressure Level at Different Times after Sensitization during Anaphylactic Shock

Statistical index	Kininogen					Blood pressure	
	before sensitization (I)	on 7th day after sensitization (II)	on 14th day after sensitization (III)	on 21st-30th day after sensitization (IV)	anaphylactic shock (V)	on 21st-30th day after sensitization (IV)	anaphylactic shock (V)
n	23	10	9	8	8	8	8
$M \pm m$	5,14±0,23	6,10 ±0,42	6,73 ±0,33	7,33 ±0,63	5,10 ±0,30	111±10	61±18
	$M_{II}-M_I$ $t=2,218$ $P<0,05$	$M_{III}-M_I$ $t=3,90$ $P<0,001$		$M_{IV}-M_I$ $t=4,91$ $P<0,001$		$M_{IV}-M_V$ $t=4,01$ $P<0,001$	

TABLE 2. Allergic Modification of Leukocytes in Percent and Antibody Titer in Rabbits at Different Times after Sensitization and during Anaphylactic Shock

Statistical index	Before sensitization (I)	On 7th day after sensitization (II)	On 14th day after sensitization (III)	On 21st-30th day after sensitization (IV)	During passive sensitization in vitro with serum obtained at height of shock (V)
n	26	11	11	8	6
$M \pm m$	11±0,8	21±2,1	34±3,2	20±2,1	9±0,8
$M_{II}-M_I$ $t=5,5$ $P<0,001$	$M_{III}-M_I$ $t=9,5$ $P<0,001$	$M_{VI}-M_I$ $t=4,8$ $P<0,001$	$M_{III}-M_{IV}$ $t=3,25$ $P<0,01$		
Titer of precipitins	—	From 1 : 40 to 1:100	From 1 : 240 to 1:2400	From 1 : 240 to 1:2400	From 1 : 100 to 1:3200

Anaphylactic shock was produced by injection of 2 ml of antigen solution into the left external jugular vein. The severity of the shock was judged from changes in the blood pressure, and the time when it reached a minimum level was regarded as the critical time in the animal's condition.

The kininogen concentration in the animals' blood serum was determined by the method of Diniz et al. [11], as modified by Paskhina and Egorova [3]. The essence of the method is that the kininogen of the blood serum is split enzymically (by means of trypsin) to bradykinin. The bradykinin concentration is determined by a biological method. The kininogen level is judged from the quantity of bradykinin formed during treatment of the serum.

The serum kininogen concentration, like the other indices, was determined several times: before the experiment, on the 7th and 14th days after sensitization, and on the day of provocation of anaphylactic shock – before injection of the reacting dose and at the time when the blood pressure had fallen to a minimum.

EXPERIMENTAL RESULTS

Sensitization of the animal led to a gradual increase in the blood kininogen concentration (Table 1). In the early stages after sensitization (7th day) an appreciable elevation of the kininogen level over its initial value was observed. As sensitization of the animal increased, as was shown by the antibody titer and the percentage of modified leukocytes (Table 2), the kininogen concentration continued to increase (14th day). Hence, during the first two weeks after sensitization, a parallel increase in the titer of precipitation and of precipitins and in the percentage of destroyed leukocytes, as well as in the kininogen level was observed in the present experiments. Later (21st-30th days after sensitization), the kininogen concentration still remained high, the antibody concentration was substantially unchanged, while the percentage of modified leukocytes had fallen considerably.

After injection of the reacting dose of antigens all the animals developed shock, varying from mild to severe. The mean values of the blood pressure before and after the reacting injection are given in Table 1. In one case the animal died at the height of the shock. In one rabbit the pressure fell almost to zero, and the animal temporarily stopped breathing. It is interesting to note that in no case was the antibody titer lowered. The kininogen concentration, on the other hand, was sharply reduced in all cases. Only in one rabbit, with the mildest course of shock, did the kininogen level fall very slightly. The decrease in kininogen concentration was evidently due to its utilization for bradykinin formation.

In anaphylactic shock the sensitizing property of the serum was maintained. Leukocytes passively sensitized with this serum behaved during contact with antigen just as in the control experiments (Table 2). The percentage of allergic modifications of the leukocytes immediately after the onset of anaphylactic shock could not be detected because of the leukopenia: the number of leukocytes during shock fell on the average by 90%.

Hence, whereas the precipitin titer was substantially unchanged and did not reflect the developing state of desensitization, the serum kininogen concentration can act in this respect as a reliable test. It can be argued that the state of desensitization was also adequately reflected by the lowering of the level of allergic modification of leukocytes passively sensitized with the serum of animals in a state of anaphylactic shock.

It can be concluded from these results that the kininogen level is definitely dependent on immunoallergic changes in the body and that it plays an important role in the pathogenesis of anaphylactic shock. Evidence of this is given by the increase in kininogen concentration, along with changes in other indices of sensitization, as the result of specific action (injection of antigen) and the marked decrease in its concentration in anaphylactic shock.

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