EFFECT OF TEMPERATURE OF INCUBATION WITH ANTISERUM ON INHIBITION OF PASSIVE ANAPHYLACTIC REACTIONS BY γ-GLOBULIN

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As a result of incubation of antiserum with γ-globulin at 4°, its anaphylactogenicity (in passive anaphylaxis reactions) is reduced more than after incubation at 37°. The greatest difference is found after incubation for 2 h.

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The inhibitory effect of γ-globulin on passive anaphylaxis has been demonstrated by several workers [3-10]. Little information is yet available on the mechanism of this action of γ-globulin [3, 4, 8, 10]. The view is held [5] that the antiallergic action of γ-globulin is based on physicochemical processes between antibodies and nonspecific γ-globulin, as a result of which the intensity of the anaphylactic phenomena is reduced.

In the present investigation the effect of the temperature of incubation of antiserum with γ-globulin on inhibition of passive anaphylaxis by the γ-globulin was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 86 guinea pigs weighing 250-300 g. A production batch of γ-globulin prepared at the Ufa Research Institute of Vaccines and Sera from human blood obtained at artificial abortion operations was used. The preparation contained 9.21% protein and was electrophoretically homogenous. Serum of rabbits immunized with alum-precipitated crystalline bovine serum albumin (BSA) was used as the source of antibodies. This serum also was used as antigen for passive anaphylactic phenomena: passive cutaneous anaphylaxis (PCA) by Ovary's method [11] and passive anaphylaxis in vitro [9].

Ovary's phenomenon was reproduced by injecting a mixture of rabbit immune serum against BSA with γ-globulin or with physiological saline (control) intradermally into guinea pigs. The control and experimental mixtures were injected into symmetrical areas of the skin of each animal. An intravenous injection of 1% Evans' blue solution and antigen was given to the animals 3-4 h after intradermal injection of the immune serum. Nonspecific staining of the injected areas of skin was not observed. The reaction was read 30 min after injection of the antigen and its intensity was assessed by the formula:

$$\frac{D_1 + D_2}{2} \cdot K,$$

where D_1 and D_2 are mutually perpendicular diameters of the stain (in mm), and K the degree of staining as shown by Ovary's scale [11].

Passive anaphylaxis in vitro was produced by heating a segment of ileum of an intact guinea pig at 37° in immune serum diluted with Krebs' solution. After rinsing, the piece of intestine was suspended in the bath of a Schultz – Dale apparatus, in which the contractions of the intestine were recorded immediately after adaptation on the smoked drum of a stationary kymograph. Contractions of two adjacent segments of ileum (experimental and control) were recorded simultaneously and under identical conditions. The experiments were duplicated: the bath used in the first experiment for the experimental segment was used

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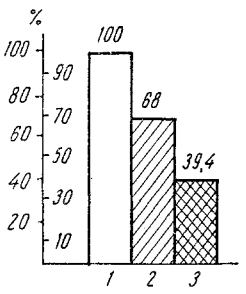

Fig. 1. Effect of γ-globulin on PCA in relation to temperature of incubation with antiserum. 1) Control (antiserum without γ-globulin); 2) incubation at 37°; 3) incubation at 4°. Ordinate, intensity of PCA (in percent).

in the second for recording the contractions of the control segment, and vice versa. Before addition of the antigen to the experimental and control baths, the reaction of the segments of ileum to histamine was determined, a "histamine scale" was obtained, and the anaphylactic contracture was measured in histamine equivalents.

The intensity of the anaphylactic phenomena in the experimental segment was expressed as a percentage of the control. The significance of the differences between compared values was determined by the X criterion [2].

EXPERIMENTAL RESULTS

In the experiments on 9 guinea pigs mixtures of antiserum with γ-globulin and with physiological saline (control) were divided in half, one half being kept for 60 min in an incubator (37°), and the other half in a refrigerator (4°). After returning to room temperature, the mixtures were injected intradermally into guinea pigs. After injection of the reacting dose of antigen with dye, the mixture of γ-globulin with antiserum kept at 4° produced a less intensive cutaneous reaction than the mixtures taken from the incubator (Fig. 1). With an increase in concentration of the preparation in the mixture, the intensity of Ovary's phenomenon diminished, and the difference between the intensities of reactions produced by mixtures incubated at 37° and 4° increased.

In the experiment on 19 guinea pigs the mixtures were incubated at the two different temperatures for 30, 60, and 120 min. The difference between the intensities of the cutaneous reaction in response to injection of mixtures kept at 37° and 4° was least after incubation for 30 min and greatest after incubation for 120 min (Table 1).

Keeping the mixtures at 4° for 12 and 24 h did not increase the inhibitory action of γ-globulin on the PCA compared with exposure for 2 h.

The next experiments used the model of passive anaphylaxis in vitro. Mixtures of antiserum with γ-globulin and Kreb's solution (control) were kept for 30 min in the incubator or refrigerator, after which adjacent segments of guinea pig ileum were sensitized in the control and experimental mixtures (after returning to room temperature) and their reaction to antigen was recorded. Investigations on 48 segments of intestine of 13 guinea pigs showed that γ-globulin definitely inhibited the passive anaphylactic contracture. No relationship was found between this effect of the preparation and the temperature of its reaction with antiserum during incubation for 30 min. When the time of contact between antiserum and γ-globulin was increased to 60 min, the inhibitory effect of γ-globulin in the mixture kept at 4° was much stronger than in that kept at 37° (Table 2).

The final experiments on 24 segments of ileum from 7 guinea pigs showed that the amplitude of the contracture was independent of the temperature of preliminary incubation of the immune serum (without γ-globulin).

TABLE 1. Effect of γ-Globulin on PCA in Relation to Temperature and Duration of Incubation with Antiserum

Incubation time (in min)	Amplitude of contracture (in %)			Significance of differences using X criterion		
	control (A)	expt. at 37° (B)	expt. at 4° (C)	B < A	C < A	C < B
30	100	29	17	> 99,5%	> 99,5%	not significant
60	100	68	39,4	99—99,5%	> 99,5%	95—97,5%
120	100	43,9	21,9	> 99,5%	> 99,5%	95—97,5%

TABLE 2. Effect of γ-Globulin on Passive Anaphylaxis in Vitro Depending on Temperature and Duration of Incubation with Antiserum

Incubation time (in min)	Intensity of reaction (in %)			Significance of differences using X criterion		
	control (A)	expt. at 37° (B)	expt. at 4° (C)	B < A	C < A	C < B
30	100	24	19,7	95—97,5%	> 99,5%	
60	100	50	8	95—97,5%	> 99,5%	95—97,5%

The results of these experiments suggest that interaction between antiserum and γ-globulin before contact with sensitized tissues (skin, intestine) plays an important role in the mechanism of the effect of γ-globulin on passive anaphylactic phenomena. A consequence of this interaction is weakening of the manifestations of the anaphylactic process, especially of its "pathophysiological" stage [1]. Temperature clearly has an essential role in this phenomenon.

LITERATURE CITED

1. A. D. Ado, in: Textbook of Pathological Physiology in Several Volumes [in Russian], Vol. 1, Moscow (1966), p. 374.
2. I. P. Ashmarin and A. A. Vorob'ev, Statistical Methods in Microbiological Investigations [in Russian], Leningrad (1962), p. 42.
3. V. A. Strigin, Abstracts of Proceedings of the Third Ukrainian Conference of Pathophysiologists [in Russian], Odessa (1966), p. 192.
4. V. A. Strigin, in: Problems in Allergy [in Russian], L'vov (1967), p. 123.
5. R. Binaghi et al., Immunology, 5, 204 (1962).
6. G. Biozzi et al., J. Immunol., 82, 215 (1959).
7. B. N. Halpern, Presse Med., 69, 1991 (1961).
8. B. N. Halpern and O. L. Frick, J. Immunol., 88, 683 (1962).
9. B. N. Halpern et al., C. R. Soc. Biol., 153, 7 (1959).
10. J. L. Mongar and H. O. Schild, J. Physiol. (London), 150, 546 (1960).
11. Z. Ovary, Internat. Arch. Allergy, 14, 18 (1959).