stances contractions appeared almost at the peak of the oscillations (Fig. 3, frequency 1.2 Hz). The amplitude of contractions increased considerably during stimulation with a high frequency, when contractions developed before the onset of oscillations (Fig. 3, frequency 2.6 Hz).

The decrease in amplitude of the contractions at the peak of the oscillations in the isovolumic guinea pig heart and in myocardial preparations from patients with heart diseases is thus identical and arises most probably on account of a decrease in the Ca^{2+} inflow into the cells [1]. If oscillations of diastolic tension develop in the patients' heart *in vivo*, they may reduce the stroke volume in cases of arrhythmia when premature contraction arises at the peak of oscillation. This may be the reason why a decrease in cardiac output is observed in patients with rheumatic heart disease and atrial fibrillation during physical function tests [3].

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STUDY OF SPASTIC REACTIONS OF THE SMOOTH MUSCLE OF THE LUNGS TO SOLUBLE IMMUNE COMPLEXES WITH MICROBIAL ANTIGENS

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Many authorities are of the opinion that soluble immune complexes (SIC), formed in some cases by autoantibodies and antigens (AG) of lung tissue [1, 4], and in other cases by soluble microbial AG and antibodies (AB) of nonreagin nature [2], participate in the pathogenesis of infectious-allergic bronchial asthma. It is considered that SIC induce an inflammatory reaction of Arthüs type in the lungs. It has also been shown experimentally that SIC may have a spasmogenic action [6, 8]. The mechanism of this phenomenon is largely unexplained. In the modern view the aggressive manifestations of SIC in the lungs are effected through a complement system [5]. The view is held that SIC act directly on smooth muscle [3]. The question of the different classes of immunoglobulins (Ig) to which the specific AB forming aggressive SIC belong likewise remains unclear.

The aim of the present investigation was to study the mechanism of the spasmogenic action of

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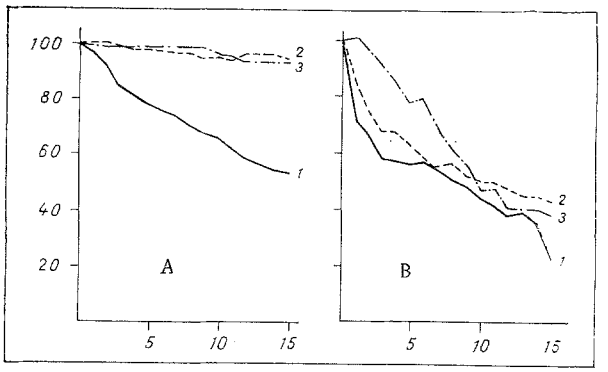

Fig. 1

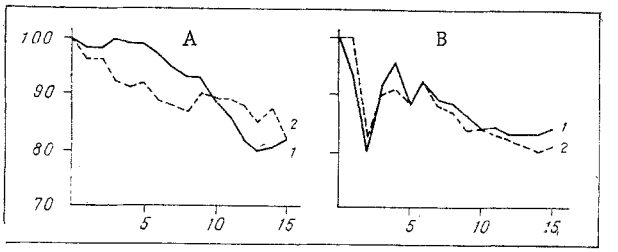

Fig. 2

Fig. 1. Action of AS-AG SIC on smooth muscle of bronchi (A) and blood vessels (B) of IL. Abscissa, time after end of injection of immune complex (in min): ordinate: A) change in amplitude of respiration, B) change in rate of perfusion (in % of initial level). 1) Immune complexes; 2) immune complexes without complement; 3) immune complexes after premedication with diphenhydramine.

Fig. 2. Action of SIC of different composition on smooth muscle of bronchi (A) and blood vessels (B) of IL. 1) Immune complexes with IgG; 2) immune complexes with IgAG. Remainder of legend as to Fig. 1.

SIC with microbial AG on the smooth muscle of the bronchi and blood vessels and also to determine the class of AB which form these SIC by using isolated lungs (IL) of intact guinea pigs as the test system.

EXPERIMENTAL METHOD

Experiments were carried out on 140 guinea pigs; 60 animals were sensitized to brucellas of vaccine strain VA-19 by subcutaneous injection of 2 billion living bacterial cells. Complement-fixing AB against soluble homologous AG, consisting of brucellas of strain VA-19 disintegrated by ultrasound, were determined 30 and 60 days later in the blood serum and the equivalent AG—AB ratio was established. Immunoglobulins of different classes were obtained by fractionation of serum on a column with Sephadex G-200. SIC in an eightfold excess of AG were prepared by the use of whole serum or different fractions of AB and soluble AG. The mixture of AG and AB was kept for 2 h at 37°C and for 18 h at 4°C.

The action of SIC was studied on the IL of 70 intact animals. The IL preparation was obtained as follows: The animals were killed by intravenous injection of hexobarbital or pentothal (0.1 mg/g body weight), with simultaneous injection of 800 units of heparin. Thoracotomy was then performed and the pulmonary artery and trachea cannulated. The lungs were removed and placed in an airtight chamber with thermostatically controlled temperature (38°C), connected to an artificial respiration apparatus. The vessels of the IL were perfused with Tyrode solution made up in 6% dextran with the addition of 2% gelatin. In the course of 20 min the vascular system of the IL was thoroughly rinsed free from blood. SIC in a volume of 1 ml was injected into the perfusion flow. The response of the vessels was assessed as the change in the number of drops of perfusion fluid flowing from the IL in 1 min. Spasmogenic substances in the perfusion fluid were determined by a biological method, using pharmacologic antagonists. Bronchospasm was judged from the decrease in amplitude of respiration of the IL recorded by means of an original respiratory temperature-sensitive element and a 4ÉÉG-1 electronencephalograph during the first 15 min after injection.

EXPERIMENTAL RESULTS

The separate injection of 0.5 ml of antiserum (AS) with AB titer of 1:80 or of 0.5 ml soluble AG into the perfusion flow of the IL caused no change in the amplitude of respiration or the rate of perfusion. In response to the action of AS—AG SIC bronchospasm developed and, at the same time, the lumen of the pulmonary vessels was reduced (Fig. 1). The perfusion fluid contained histamine, evidence of the participation of this mediator in the spastic reaction. Preliminary injection of the histamine antagonist diphenhydramine (1 mg) into the perfusion flow prevented the spastic action of the SIC on the bronchi. Consequently, endoge-

nous histamine must be considered to be the effector stage in the mechanism of action of SIC on the bronchial smooth muscle in these experiments.

To study the role of complement, IL were treated with AS–AG SIC in which the AS was deprived of complement by heating to 56°C for 30 min. As Fig. 1 shows, no response of the bronchi occurred. In addition, no histamine was found in the perfusion fluid. It is evident that activation of the complement system is essential for the liberation of endogenous histamine under the influence of SIC. The most likely source of histamine in IL can be taken to be the tissue mast cells, for other possible sources (in particular, basophils) had been removed from the pulmonary vessels during perfusion. The mechanism of the spastic response of the blood vessels of IL to SIC was evidently of a different character. The absence of complement did not prevent narrowing of the lumen of the vessels. In addition, premedication with diphenhydramine did not abolish the vasoconstrictor response to injection of SIC (Fig. 1). SIC may have a direct action on the smooth muscle of blood vessels.

To determine the class of immunoglobulins to which the AB participating in the formation of SIC with spasmogenic activity belonged, gel-chromatographic fractions of γ-globulins containing IgM, IgM mixed with IgG (IgAG — the ascending part of fraction II of the elution profile), and IgG were used (Fig. 2). It was found that IgM fraction did not contain complement-fixing AB against soluble AG. A mixture of that particular fraction, AG, and complement (control) had no spastic action on the IL preparation. The IgAG and IgG fractions contained specific complement-binding AB in a titer of 1:8, by contrast with whole AS, in which the titer of specific AB was 1:80. Administration of SIC containing AB from these fractions and complement (a commercial preparation in a working dose for the complement fixation test) into the IL perfusion flow had a spastic action both on the bronchi and on the blood vessels of IL (Fig. 2). Differences from the control were significant ($0.01 < P < 0.05$). Under these circumstances the effects of SIC with IgAG and with IgG did not differ significantly from one another ($P > 0.05$). Histamine was found in the perfusion fluids only after the action of SIC, but was not present in the control experiments.

Bronchospasm induced by SIC with IgAG and IgG fractions is evidence that specific IgG-AB definitely participate in this reaction. As regards AB of the IgA class, their participation in the formation of aggressive SIC is doubtful, for it is considered that IgA cannot bind component Clq of complement [7]. It can accordingly be postulated that the AB in the IgAG fraction forming biologically active SIC with AG belong to the IgG class.

The spastic action of SIC with microbial AG on the bronchi is therefore mediated through the complement system, which evidently causes degranulation of the mast cells and liberation of histamine. The vasoconstrictor response to SIC is evidently due to the direct action of the SIC on the smooth muscle of the blood vessels. The AB forming immune complexes with aggressive properties belong to the IgG class.

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