STATE OF ADRENERGIC AND CHOLINERGIC NERVOUS STRUCTURES OF THE RABBIT HEART AFTER SENSITIZATION AND ANAPHYLACTIC SHOCK

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It has been shown both experimentally and clinically by pharmacological and electrophysiological methods that the consequences of sensitization and anaphylactic shock are intensive stimulation, followed by inhibition of the function of the nervous system; in the blood this is accompanied by an increase in the concentration of biologically active substances, including catecholamines (adrenalin) and acetylcholine [1, 3, 5, 9]. With the introduction of improved histochemical methods of detection of autonomic nervous system mediators — catecholamines and acetylcholinesterase (AChE) — in the tissues it has now become possible to determine these substances quantitatively and qualitatively in different pathological states.

This paper describes a study of the morphological and functional state of adrenergic and cholinergic nervous structures of the heart in the course of sensitization and after anaphylactic shock, for we know that changes in the innervation of the heart affect its functions.

EXPERIMENTAL METHOD

Rabbits were sensitized by four subcutaneous injections of normal horse serum (each of 0.5 ml/kg body weight) with intervals of 3 days between injections. Anaphylactic shock was induced by intravenous injection of normal horse serum 14 days after the last sensitizing injection. The neurohistochemical investigations were carried out on 63 hearts from rabbits (20 sensitized, 22 anaphylactic, and 21 control) aged 2-3 months and weighing 2.0-2.5 kg. The experimental animals were divided into three groups. Group 1 consisted of rabbits whose cardiac nervous structures were studied during sensitization (after the first, second, third, and fourth sensitizing injections). Group 2 included animals in which the same structures were studied after death from anaphylactic shock. Group 3 included rabbits surviving after anaphylactic shock and killed on the 7th day after the experiment. The control group consisted of intact rabbits of the same age as the sensitized animals (one animal for every two experimental rabbits). Material for histochemical investigation during sensitization was obtained before each sensitizing injection, and after the fourth sensitizing injection — on the 14th day (before anaphylactic shock). The heart was removed immediately after breathing stopped and transferred into physiological saline, and cut into pieces measuring 1 × 1 cm, which were frozen in liquid nitrogen. Frozen sections were mounted on slides. Some sections were lyophilized and tested for catecholamines (adrenergic nerve fibers), and other sections were tested for AChE by the method of Karnovsky and Roots [1]. The density of the nervous plexuses was determined planimetrically, by the dot method [8], in which the number of dots lying above nervous structures was determined as a percentage of the number of dots in the whole field of vision.

EXPERIMENTAL RESULTS

No histochemical differences could be found in the test structures when adrenergic and cholinergic nerve fibers were studied in the hearts of the animals of group 1 and the control

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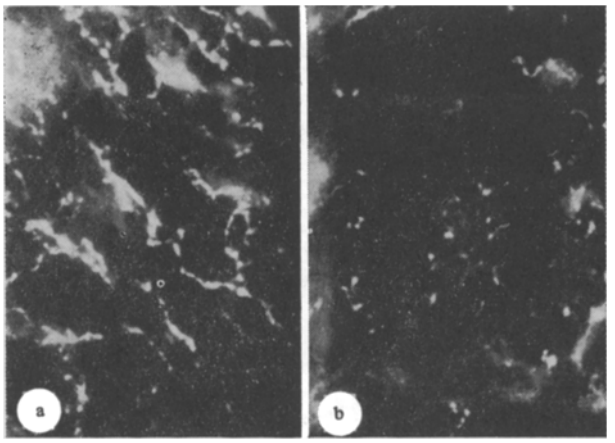

Fig. 1. Adrenergic nerve plexuses of right atrium. a) Control; b) 7th day after anaphylactic shock. Reaction for catecholamines in paraform vapor using lyophilized frozen sections. Objective 40, ocular homal 3.

group. Differences were found in the concentration of adrenergic and cholinergic structures, and these were most numerous in the atria, the right auricle, and the conducting system, in agreement with data obtained by other workers [4, 6, 8], Adrenergic nervous structures of the heart were revealed as fibers, preterminals, and terminals. Varicose expansions were found along their course. Thicker adrenergic nerve fibers, with varicose changes, were present in the auricles and atria. Adrenergic nerve fibers, their preterminal segments, and terminals interwove in the atria and formed a network (Fig. 1a). In the ventricles adrenergic structures entwined blood vessels and ran parallel to the muscle fibers of the myocardium (Fig. 2a).

Intercardiac cholinergic neurons and nerve bundles were concentrated most thickly on the dorsal surface of the coronary sulcus and in the dorsal part of the atrial septum. Cholinergic nerve fibers were oriented parallel to muscle fibers, and their terminals formed a dense plexus in the myocardium of the atria. In the myocardium of the ventricles no cholinergic nervous structures could be found (by the method which we used). A positive reaction for AChE was found along the nervous plexuses of the blood vessels of the ventricular myocardium. It was found as an exception that the number of adrenergic terminals and varicose expansions and the intensity of luminescence decreased as early as after the fourth sensitizing injections. The density of nerve fibers was reduced (compared with the control) on average to 19% in the atria and to 14% in the ventricles. An increase in autoluminescence was observed in the myocardium of the hearts of rabbits dying from anaphylactic shock (group 2) and in animals killed on the 7th day after anaphylactic shock (group 3). A characteristic feature of the adrenergic nervous structures in the heart of the animals of these groups was a focal decrease in the intensity of luminescence of efferent terminals and in the number of varicose expansions (Fig. 1b). These changes were more marked in the adrenergic nervous plexuses of the hearts of the rabbits of group 3. Compared with myocardial adrenergic structures, the intensity of luminescence and of varicosity of the terminals remained high in the corresponding perivasal structures. At the same time, autoluminescence in the perivasal zone was exhibited more strongly than in other regions of the myocardium (Fig. 2b).

Changes in the structure of the cholinergic nerve fibers were observed only on the 7th day after anaphylactic shock. They were expressed as a decrease in intensity of the histochemical reaction for AChE in nervous structures in all parts of the heart. AChE activity in the intracardiac neurons varied, and more neurons with palely stained cytoplasm in the region of the perikaryon appeared (Fig. 3). The paler staining of the terminal cholinergic branches became more clearly visible, and as a result the density of these structures was reduced to 9% in the atria compared with the control.

The changes observed are evidently interconnected with other morphological and functional disturbances in the heart. Previous investigations [2, 5, 7, 9] showed that after

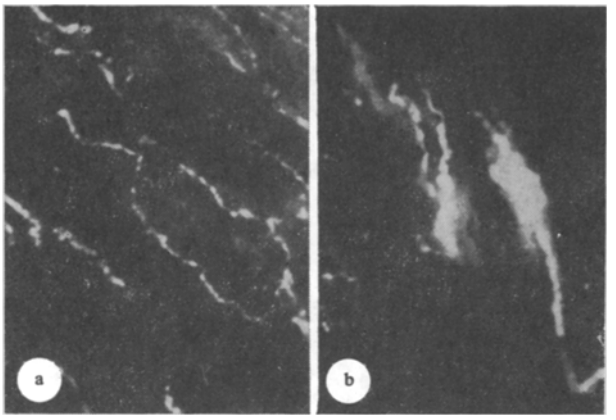

Fig. 2. Adrenergic nerve plexuses in right ventricle. Legend as to Fig. 1.

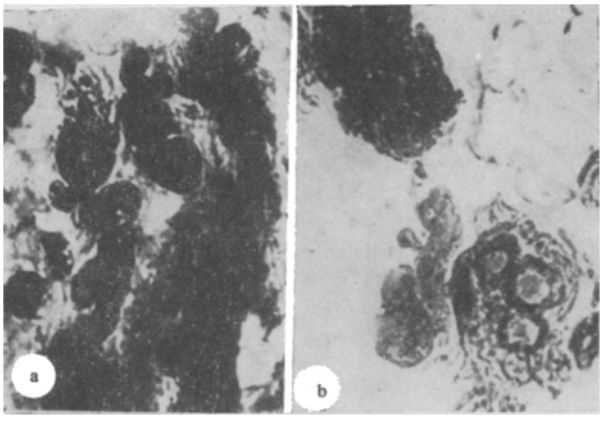

Fig. 3. Cholinergic neurons and nerves of right atrium. Reaction for AChE by Karnovsky—Roots method. Objective 40, ocular 7. Remainder of legend as to Fig. 1.

sensitization of animals and anaphylaxis, the hemodynamics in the myocardium, respiration, and synthesis of high-energy phosphorus compounds are disturbed and dystrophic changes, followed by necrotic changes, develop in the myocardium. The causes of these changes have not yet been fully explained. In recent years conclusive evidence has been obtained [9, 10, 12] that injury to intracardiac nerve ganglia and fibers leads to disturbance of the adaptive-trophic innervation of the heart and promotes the development of dystrophic changes in the myocardium. Consequently, it can be postulated on the basis of data in the literature that the morphological and functional changes detected in the myocardium during allergy are dependent to some extent on the state of its adrenergic and cholinergic nerve structures.

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INDUCTION OF BRONCHOSPASM BY MEDIATORS OF ANTIGEN-SPECIFICALLY STIMULATED LYMPHOCYTES

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Soluble mediators of cellular immunity, namely lymphokines (LK), bring about interaction between the various types of cells that participate in the immune response [3]. The number of LK described in the literature [6] is 56. The target of their action may also be cells not directly concerned with the immune response (fibroblasts, epithelial cells, platelets, erythrocytes, etc.).

No data could be found in the literature on the effect of LK on smooth muscle tissue function. Yet this problem is of both scientific and practical importance in the elucidation of the pathogenesis of the infectious-allergic form of bronchial asthma and of the asthmatic phenomena associated with respiratory infections on a wider plane. This disease is one of the most important respiratory diseases and it is usually associated with delayed-type hypersensitivity (DTH) to microbial antigens [1, 2].

This paper describes the results of a study of the action of antigen-specific and antigen-nonspecific LK on the bronchial smooth muscle of isolated guinea pig lungs.

EXPERIMENTAL METHOD

Experiments were carried out on 110 noninbred guinea pigs, in 20 of which DTH was induced against brucellas of the vaccine strain VA-19, and in another 20 against *Staphylococcus aureus* strain Cowan 1. The methods of sensitization were described previously [2]. After 1-1.5 months the development of DTH was investigated by skin tests and the blood leukocyte migration inhibition test (LMIT), and only animals giving a positive reaction were used in the subsequent experiments. All experiments on the animals were performed under open ether anesthesia. Total LK were obtained as the 24-h supernatant of lymphocytes in conical test tubes by the method in [7]. The thymus, lymph nodes, and spleen were homogenzied and a suspension containing 5×10^7 cells/ml was cultured in medium 199 with the addition of 100 µg/ml of staphylococcal or 200 mg/ml of brucella antigen (AG) (the AG were obtained by ultrasonic disintegration of the microorganisms [2]), 10% bovine serum, and 1000 I.U. each of penicillin and streptomycin. The presence of LK in the supernatant was tested by the peripheral blood LMIT in intact animals by a modified method [4]. Supernatants active in the indirect LMIT were used for investigation of the reactions of isolated guinea pig lungs (IL). Parallel with these experiments the following controls were used: 1) control of the medium for incubation of lymphocytes (MC); 2) AG in a working concentration (AGC); 3) control of supernatants without AG (LKC) and of the supernatant after incubation of lymphocytes with heterologous AG (HAGLK), at which an ultrasonic extract of *Escherichia coli* in a dose of 200 µg/ml was used. The test agents were injected into the IL perfusion flow in a volume of 3 ml.

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