PARASYMPATHETIC COMPONENT OF REGULATION OF THE KALLIKREIN–KININ SYSTEM IN EXPERIMENTAL PNEUMONIA

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The kallikrein–kinin system (KKS) plays an important role in the regulation of many of the bodily functions. Information has been obtained on its role in the pathogenesis of many diseases and pathological states, including lung diseases [6, 15].

The aim of this investigation was to study activity of the blood KKS in experimental pneumonia, with special regard to the regulatory role of the vagus nerves in the formation and destruction of kinins.

EXPERIMENTAL METHOD

Experiments were carried out on 26 rats and 53 rabbits. The state of the KKS was assessed by determining the kininogen level by the method in [13] in the modification of Paskhina et al. [10], kallikrein activity [12], concentrations of prekallikrein and kallikrein inhibitors by the method in [14], as modified by Gomazkov et al. [3], free kallikrein activity [11], and activity of kinin-destroying blood enzymes [2]. Activity of the lung kininases was determined by perfusion of the pulmonary vessels with a solution of bradykinin and determination of its concentration in the perfusion fluid draining from the lungs [8], and also on the basis of the hypotensive action of bradykinin, when injected separately into the systemic and pulmonary circulations [4]. A model of acute pneumonia was formed by Dubilei's method, by unilateral vagotomy and insertion of an infected length of catgut into the lumen of the trachea through a microtracheostomy [7]. A chronic form of disease was obtained by a modification of the method in [1]. The diagnosis was confirmed clinically and roentgenologically, and later, histologically. Since in this model of experimental pneumonia an important role is played by blockade of vagus nerve influences (unilateral vagotomy) on the lungs (which substantially modifies metabolic processes in them), the regulatory role of the vagus nerves on kinin formation and kinin destruction was investigated initially by studying KKS activity 10 min and 2 h after vagotomy and also in the first minute after electrical stimulation of the peripheral ends of the divided vagus nerves in vagotomized animals (ÉSU-1 electrical stimulator, parameters of stimulation: 10 V, 0.5 msec, 20 Hz).

EXPERIMENTAL RESULTS

It will be clear from Table 1 that after division of the vagus nerves kallikrein activity was increased in the experimental animals, whereas concentrations of kininogen and kallikrein inhibitors were reduced, evidence of activation of kinin formation [9], but the increase in the prekallikrein concentration which was found often accompanies intensification of kinin formation [5]. Similar changes also were observed 2 h after vagotomy. The general pattern of changes in components of the KKS observed after electrical stimulation of the vagus nerves pointed to a decrease in kinin forming activity compared with the changes after vagotomy.

In rabbits with experimental pneumonia kallikrein activity on the 10th-14th day of development of acute inflammation was 2.27 ± 0.19 μg/ml, significantly higher than values in the control (0.91 ± 0.04 μg/ml; $p < 0.001$). The kininogen concentration fell to 1.5 ± 0.32 μg/ml (3.32 ± 0.12 in the control; $p < 0.001$), whereas activity of the kinin-destroying enzymes of

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TABLE 1. Levels of Components of Blood KKS after Cervical Vagotomy and Electrical Stimulation of Peripheral Ends of Divided Vagus Nerves in Laboratory Animals

Parameter	Initial value	10 min after vagotomy	p	After electrical stimulation	p
Kininogen, μg/ml eq bradykin	3,68±0,13	2,46±0,16	0,001	3,27±0,14	0,01
Free kallikrein, mU/ml	12,1±1,7	30,5±2,3	0,001	11,4±2,0	0,001
Prekallikrein, μmoles BAME/ml/h	83,6±2,1	111,2±5,3	0,001	110,4±5,8	0,5
Kallikrein inhibitors, conventional units	0,92±0,04	0,53±0,04	0,001	1,02±0,04	0,001
Kininases, μg inactivated bradykinin/ml/min	1,82±0,12	3,82±0,21	0,001	3,95±0,19	0,5
Destruction of bradykinin inlungs, %	91,6±0,6	92,3±0,6	0,5	94,7±0,5	0,05

Legend. p) Significance of differences compared with control, p_1) the same, compared with values for vagotomized animals.

the blood fell to 1.28 ± 0.05 μg/ml·h (1.73 ± 0.05 μg/ml·h in the control; $p < 0.01$). Kinin destruction in the lung fell to 64.5 ± 3.8% (86.1 ± 2.2% in the control). In the group of animals with chronic inflammatory disease of the lungs, increased activity of the blood kallikrein to 1.4 ± 0.12 μg/ml ($p < 0.001$) and a decrease in the kininogen concentration to 2.18 ± 0.21 μg/ml ($p < 0.001$) were observed on the 30th-33rd days. Plasma kinin destruction was reduced under these circumstances to 0.28 ± 0.07 μg/ml·h, and its destruction in the lung was reduced to 22.2 ± 6.1 μg/ml·h ($p < 0.001$).

Thus increased activity of kinin formation was found both in experiments with isolated vagotomy and also in animals developing experimental pneumonia. Since the model of experimental pneumonia which was used included components of unilateral vagotomy and bacterial infection (*Staphylococcus aureus* P-209), activation of KKS in this case was evidently due to a combination of disturbance of the parasympathetic regulation and the response of the animal to bacterial infection. Meanwhile, in a study of activity of kinin-destroying enzymes in the blood and the kinin-activating capacity of the lungs, different results were obtained when vagotomy was performed separately and during the development of experimental pneumonia. Whereas in the first series of experiments there was an increase in kininase activity in the blood but no change in kininase activity in the lungs, in the second series these parameters showed a fall. Reduction of the kininase activity in the blood and lungs during endotoxic shock and in other diseases accompanied by bacteriemia has been found by several other investigators [15].

The reduction of kininase activity in the blood and lungs, observed in experimental pneumonia, is thus probably due to bacterial action and is an important factor leading to the accumulation of high concentrations of kinins in the blood.

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CYCLIC NUCLEOTIDES IN EXPERIMENTAL GLAUCOMA

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There is evidence in the literature that the sympathetic nervous system is involved in the pathogenesis of glaucoma [2, 3, 10, 13]. However, these data are largely contradictory.

The concept of the role of chronic activation of the β-receptor apparatus of the eye as an important pathogenetic mechanism of glaucoma development was put forward by Kryzhanovskii et al. [4]. Their investigations showed that blockade of the sympathetic effect with lithium hydroxybutyrate [7], like β-receptor blockade, can prevent the development of experimental glaucoma, whereas α-receptor blockade may facilitate its development [4, 5]. It is an interesting fact that noradrenalin, which activates primarily α-receptors, can weaken the galucomogenic effect of adrenalin, which activates mainly β-receptors [6].

We know that the hormonal and neurotransmitted signal from β-adrenoreceptors is realized by cyclic AMP (cAMP). The state of β-reception can therefore be judged to some extent by the intracellular cAMP level. Changes in cyclic nucleotide (cAMP and cGMP) concentrations have been found in the aqueous humor of glaucoma patients. However, determination of the cyclic nucleotide levels in the aqueous humor is not sufficient to allow the state of this system to be judged in the eye tissues.

The aim of this investigation was to study concentrations of cyclic nucleotides cAMP and cGMP in different parts of the eyes of rabbits with experimental glaucoma.

A model of experimental glaucoma developed in the Academician V. P. Filatov Odessa Research of Eye Diseases [9], induced by chronic intravenous injection of adrenalin, was used.

EXPERIMENTAL METHOD

Experiments were carried out on "White Giant" rabbits about 2 years old. The control animals were of the same age. Glaucoma was induced by intravenous injection of 0.1 ml of 1:1000 adrenalin solution on alternate days for 3 months [9]. The intraocular pressure (IOP) was measured tonometrically daily. Material was taken for biochemical study 1.5 months after the beginning of adrenalin injections, when the initial changes in IOP had appeared, and 1.5 years after cessation of the 3-month periods of adrenalin injections, when the animals had developed persistent and advanced glaucoma. Weighed samples of eye tissues, put into test tubes with 2 ml of ethanol, purified with silver nitrate and converted into the absolute form, were homogenized with quartz sand and centrifuged at 2000 rpm for 15 min. The residue was washed with 1 ml of alcohol and recentrifuged under the same conditions. The pooled supernatent — an alcoholic tissue extract — was evaporated at 55°C. Before determination of cyclic nucleotides the evaporated extracts were diluted with Tris-EDTA buffer, pH 7.5 [11].

The cAMP and cGMP concentrations were determined in tissues of the retina, the vascular coat of the eye, ciliary body, and iris by radioimmunoassay, using commercial kits ("Amersham International," England). Radioactivity was counted on a RackBeta scintillation counter (LKB, Sweden). The results were subjected to statistical analysis.

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