Kallikrein-Kinin, Proteolytic, and Antiproteolytic Systems of the Lymph During Febrile Reactions

M. M. Minnebaev, F. I. Mukhutdinova, and R. M. Minnebaev

UDC 616-092:612.55]-07:616.4-008.831:577.152.34

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 6, pp. 577-579, June, 1994
Original article submitted November 16, 1993

Experiments on rabbits showed that, in the course of a febrile reaction, alterations in the activities of the kallikrein-kinin, proteolytic, and antiproteolytic systems of the lymph and blood are of a systemic nature, i.e., they occur in all components of humoral transport, attesting to the close interrelationship and unity of these systems in body fluids. Depending on the duration of the febrile reaction, the time course of these systems' components was characterized by phasic increases and decreases in their activity.

Key Words: *kallikrein-kinin system; lymph; febrile reaction*

Given the constancy of transcapillary exchange among components of the kallikrein-kinin system (blood - tissue fluid [and cellular elements of the reticuloendothelial system of internal organs] - lymph - blood), alterations in its activity during a febrile response should be expected to affect not only the blood but the lymph as well. The resorptive and transporting functions of the lymphatic system may in turn mediate alterations in levels of individual components of the kallikrein-kinin (KK) system in the blood. On the other hand, there is evidence that the kinin system plays an important role in the central mechanisms of febrile reactions [4]. In our view, a better insight into the involvement of kinins in febrile reactions of various duration can be gained by undertaking a comparative analysis of alterations that occur during such a reaction in components of the KK, proteolytic, and antiproteolytic systems of the lymph (drained from various body areas) and of the peripheral blood.

MATERIALS AND METHODS

For the study, 76 Chinchilla rabbits weighing 2.5 to 4.2 kg were used. A febrile reaction was produced by intravenous injection of a lipopolysaccharide pyrogen (Pyrogenal) as described previously [8]. Lymph was collected from the thoracic lymphatic duct, the postnodal portion of the hepatic lymphatic duct, and the intestinal lymphatic trunk; blood was taken from the femoral vein. At different times during febrile reactions of varying length, lymph and blood samples were assayed for kininogen [9], prekallikrein, fast- and slow-reacting kallikrein inhibitors (FKI, SKI) [3], kallikrein [12], kininase activity [12], free kinins [11], total proteolytic activity (TPA) [1], total antiproteolytic activity (TAPA) [7], α-2-macroglobulin (α-2-MG) [2], and α-1-inhibitor of proteinases (α-1-PI) [13]. Lymph and blood were collected under conditions preventing spontaneous production and degradation of kinins. As controls, rabbits injected with apyrogenic physiological saline were used. Euthanasia after the tests was performed with a lethal dose of a narcotic substance.

RESULTS

As can be seen in Table 1, the lymph samples contained all KK system components required for the formation and inactivation of kinins. Since kinins are normally incapable of penetrating through the vessel wall in an active state [16], their pres-

Department of Pathological Physiology, S. V. Kurashov Medical Institute, Kazan'. (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0006-0576$12.50

ence in the lymph is an indication that basal kininogenesis is proceeding in the latter at a rate that is lower than that in the blood because of the lower activity of the components responsible for kininogenesis. The lymph KK system is functionally complete in that it is capable of responding to a variety of factors. Also, it has been shown that when the rate of kininogenesis in the plasma increases or decreases, so does the rate of kininogenesis in the lymph [14].

Alterations in the activitiy of the KK, proteolytic and antiproteolytic systems during febrile reactions are of a systemic nature, i.e., they occur in all components of humoral transport, which attests to the close interrelationship and unity of these systems in body fluids. However, depending

TABLE 1. Lymph and Blood Levels of Components of the Kallikrein–Kinin and Antiproteolytic Systems and of Total Proteolytic Activity During Febrile Reactions of Varying Duration ($M \pm m$)

Parameter	Control rabbits	Number of Pyrogenal injections							
		one		three			five		ten
		after 2.5 - 3 h	after 5 - 5.5 h	day 4	day 6	day 10	day 4	day 6	day 10
Thoracic duct									
Kininogen[1]	5.4±0.3	5.4±0.1	5.6±0.3	5.7±0.2	5.8±0.2	5.7±0.2	4.7±0.2	4.8±0.3	4.2±0.2*
Free kinins[2]	1.2±0.2	2.2±0.2*	1.9±0.2*	2.6±0.2*	1.4±0.1	1.1±0.2	2.6±0.2*	1.2±0.2	1.8±0.2*
Prekallikrein[3]	38.1±3.5	39.1±2.9	35.5±2.8	36.0±3.7	38.4±1.9	41.4±2.3	27.3±1.9*	39.1±2.1	29.2±1.3*
SKI[3]	1.1±0.1	1.1±0.1	0.6±0.1*	0.7±0.1*	1.1±0.1	1.2±0.1	1.3±0.2	1.3±0.1	0.7±0.1*
FKI[3]	3.5±0.2	3.0±0.3	3.5±0.3	2.9±0.2	3.3±0.2	2.6±0.2	3.7±0.2	3.7±0.2	2.2±0.2*
Kallikrein[1]	1.6±0.5	1.7±0.1	1.8±0.2	1.3±0.1	1.0±0.1*	1.6±0.1	0.9±0.1*	1.7±0.1	1.1±0.1*
Kininase activity[4]	0.09±0.01	0.11±0.01*	0.09±0.01	0.07±0.01	0.09±0.01	0.11±0.01*	0.12±0.01*	0.10±0.01	0.07±0.01*
α–2–MG[5]	1.4±0.1	2.0±0.1*	1.9±0.2*	2.0±0.1*	1.3±0.1	1.4±0.1	2.1±0.1*	1.1±0.1*	1.0±0.1*
α–1–PI[5]	30.0±1.5	41.5±1.3*	37.2±2.1*	39.8±2.3*	31.6±2.0	31.9±2.0	42.1±1.9*	23.2±1.5*	25.2±1.8*
TAPA[6]	1.0±0.1	1.4±0.1*	1.4±0.1*	1.4±0.1*	1.0±0.1	1.0±0.1	1.5±0.1*	0.7±0.1*	0.7±0.1*
TPA[7]	1.1±0.1	1.6±0.1*	1.7±0.1*	1.9±0.1*	1.0±0.1	1.1±0.1	2.1±0.1*	1.1±0.1	1.7±0.1*
Hepatic lymph									
Kininogen[1]	5.7±0.4	5.7±0.5	3.7±0.3*	5.3±0.4	5.5±0.7	5.2±0.5	4.0±0.4*	5.4±0.8	3.6±0.3*
Prekallikrein[3]	39.1±3.0	36.4±2.9	26.6±3.2*	34.4±2.7	38.7±2.5	36.0±2.3	28.4±1.7*	37.3±1.9	27.9±1.7*
SKI[3]	1.6±0.2	1.9±0.1	0.9±0.1*	1.0±0.1*	1.3±0.1	1.4±0.1	1.6±0.1	1.6±0.2	0.9±0.1*
FKI[3]	5.0±0.3	3.3±0.3*	3.3±0.3*	2.9±0.2*	4.1±0.1	4.2±0.5	4.3±0.4	5.0±0.3	2.2±0.2*
Kallikrein[1]	2.6±0.3	2.3±0.2	3.2±0.2*	2.7±0.3	2.5±0.3	2.5±0.2	1.8±0.1*	2.7±0.2	2.5±0.3
Kininase activity[4]	0.09±0.01	0.12±0.01*	0.07±0.01	0.08±0.01	0.08±0.02	0.11±0.01	0.14±0.01*	0.09±0.01	0.05±0.01*
TPA[7]	1.0±0.1	1.8±0.1*	1.9±0.1*	1.9±0.2*	1.1±0.1	1.1±0.1	2.0±0.1*	1.0±0.1	2.2±0.2*
Intestinal lymph									
Kininogen[1]	3.2±0.3	3.5±0.4	3.6±0.3	3.5±0.6	4.1±0.5*	4.1±0.7*	3.5±0.6	4.1±0.3*	2.1±0.2*
Prekallikrein[3]	26.7±1.9	28.1±1.8	30.1±2.5	29.2±2.7	30.0±2.9	28.9±2.6	22.1±1.8*	28.2±1.7	22.5±1.6*
SKI[3]	1.1±0.1	1.0±0.1	0.7±0.1*	1.0±0.1	1.0±0.1	1.2±0.1	1.2±0.1	1.2±0.1	0.5±0.1*
FKI[3]	3.2±0.2	3.6±0.3	2.9±0.1	2.6±0.3	3.1±0.4	3.6±0.5	3.1±0.2	3.0±0.4	2.1±0.2*
Kallikrein[1]	1.0±0.1	1.1±0.1	1.1±0.1	0.9±0.1	1.1±0.2	1.0±0.1	1.6±0.1*	1.1±0.1	1.9±0.1*
Kininase activity[4]	0.07±0.09	0.10±0.01*	0.08±0.01	0.06±0.01	0.06±0.01	0.07±0.01	0.07±0.01	0.07±0.01	0.05±0.01*
TPA[7]	1.3±0.1	2.0±0.1*	2.1±0.2*	1.9±0.2*	1.2±0.1	1.2±0.1	2.4±0.1*	1.3±0.1	1.7±0.2*
Blood plasma									
Kininogen[1]	6.5±0.3	6.7±0.2	7.0±0.2	6.4±0.2	6.9±0.2	7.0±0.1	5.8±0.2*	6.1±0.2	7.0±0.2
Free kinins[2]	2.2±0.1	2.2±0.3	2.3±0.2	3.3±0.3*	2.0±0.1	1.8±0.1	3.8±0.2*	2.3±0.2	2.6±0.2
Prekallikrein[3]	64.5±2.6	66.3±2.6	67.9±2.3	66.7±2.2	54.5±1.9*	64.5±1.9	57.8±1.9*	56.8±2.8*	55.5±2.2*
SKI[3]	2.4±0.2	2.2±0.2	2.7±0.1	1.6±0.1*	2.7±0.2	2.6±0.2	1.6±0.1*	1.8±0.1*	1.7±0.1*
FKI[3]	6.5±0.2	5.9±0.2	6.5±0.3	5.0±0.3*	4.9±0.2*	6.6±0.3	6.3±0.2	6.5±0.3	6.4±0.4
Kallikrein[1]	1.3±0.1	1.3±0.1	1.2±0.1	1.7±0.2*	2.0±0.2*	1.0±0.1*	1.0±0.1*	2.1±0.2*	1.1±0.1
Kininase activity[4]	0.12±0.01	0.11±0.01	0.11±0.01	0.14±0.01	0.14±0.01	0.12±0.01	0.13±0.01	0.09±0.01*	0.09±0.01*
α–2–MG[5]	2.1±0.1	2.7±0.2*	2.7±0.2*	3.2±0.2*	2.1±0.1	2.2±0.1	3.0±0.3*	1.4±0.2*	1.6±0.2*
α–1–PI[5]	46.5±1.8	56.9±2.6*	55.5±2.1*	54.9±1.9*	43.9±1.7	42.2±2.1	54.4±1.7*	36.3±1.9*	34.1±1.7*
TAPA[6]	1.4±0.1	1.8±0.1*	2.1±0.1*	2.4±0.2*	1.4±0.1	1.5±0.1	2.0±0.1*	1.1±0.1	1.0±0.1*
TPA[7]	1.9±0.2	2.4±0.2*	2.8±0.2*	2.6±0.2*	1.5±0.1	2.0±0.2	2.9±0.1*	1.8±0.1	2.9±0.1*

Note. An asterisk denotes reliability vis–a–vis control. Superscripts: 1) μg/eq bradykinin/ml; 2) nmol/liter bradykinin; 3) μmol arginine/liter per min; 4) μg/eq bradykinin/ml/ min; 5) μmol/liter; 6) g inactivated trypsin/liter; 7) μmol/liter/hour.

on the duration of the febrile reaction, the time course of these systems' components was found to be characterized by phasic changes - increases and decreases in their activity. We identified four types of responses shown by the KK system of the lymph and blood in fever.

Type I: "the usual" proportional activation of the KK system, which characterizes the maintenance of biochemical equilibrium in the system itself and which can probably be regarded as a compensatory response [5]. This activation was manifested in a lowered kininogen level, in elevations of prekallikrein and of the enzymatic activity of kallikrein and kininases, and in enhanced formation of free kinins. Such changes were observed after one or three Pyrogenal injections. Early during the febrile reaction, they were marked most strongly in the hepatic lymph and less so in the thoracic duct lymph. In addition, elevations of both TPA and TAPA and of α-2-MG and α-1-PI were noted.

The KK system and febrile reaction may be activated by many factors. We believe that the biologically active substances released during fever increase vascular permeability and promote, on the one hand, the penetration of plasma proteins into the extravascular space where tissue kinins are then activated, and, on the other hand, the exit of intracellular enzymes into the blood and lymph with a resultant elevation of the proteolytic activity of these body fluids. In their turn, the proteolytic enzymes increase vascular permeability both directly, by acting on the vessel wall endothelium, and indirectly, by inducing the formation and release of permeability transmitters [8]. The KK system may be activated as a consequence of hypoxia, metabolic acidosis, or mobilization of lysosomal enzymes. Acidosis, which may arise during fever as a result of the accumulation of incompletely oxidized metabolic products, causes reversible inhibition of kininase and an increase in kininogenase activity, and this, too, creates conditions favoring enhanced activity of the KK system [6]. Kinins have been shown to be produced from kininogen in the presence of leukocytes. The membrane of these cells carries a plasminogen activator converting plasminogen to plasmin, which then stimulates the generation of free kinins from kininogen. This mechanism operates most efficiently during endothermy [15]. It is possible that the activation of kinin generation in fever is accompanied by inhibition of kinin-degrading processes, which may be an additional mechanism whereby the concentration of active kinins is raised in the lymph and blood.

The KK system is at all times under the influence of the autonomic nervous system, whose sympathetic part stimulates kininogenesis and whose parasympathetic part inhibits it [10]. Since both parts of this system may be activated in the course of a febrile reaction (depending on its stage), we believe that not only enhancement but also some inhibition of kininogenesis may occur during such a reaction. This can probably explain why at some times during our study we did not observe changes in the lymph and blood that usually occur in the classic form of KK system activation, namely a fall in kininogen and boost of kininase activity.

Type II: activation of the kinin system with a disturbance of quantitative relationships among its components (decreases in prekallikrein and kininogen levels in the thoracic duct lymph, hepatic lymph, and venous blood paralleled by a fall in kallikrein concentration), which could be taken as evidence that the regulatory links in this system were being "severed" and that physiological reactions were turning into pathogenetic ones. Such changes were observed shortly after five injections of the lipopolysaccharide (Pyrogenal) and were more strongly marked in the lymph than in the blood.

Type III: a combination of type II with some depletion of the KK system and of the intrasystemic mechanisms of compensation for intensified kinin generation and with partial restoration of kininogenesis (elevation of kininogen in the intestinal lymph and decreases in TAPA and in levels of proteinase inhibitors in the thoracic duct lymph). This type of response was observed on the 10th day after a fever lasting five days.

Type IV: the development of "deficits" of the major components of the KK system as a result of diminished kinin generation, as was indicated by falls in the content of all components of the kinin and antiproteolytic systems, particularly in the lymph, after ten Pyrogenal injections. Yet kininogenesis still proceeded at minimal rates that differed in different regions (the concentration of free kinins rose in the thoracic duct lymph while that of kallikrein rose in the intestinal lymph).

The results of our study suggest that phasic changes in the KK system's activity in body fluids are a link in the chain of events involved in the complex pathogenesis of disturbances in homeostasis and the functional activity of organs and systems during a febrile reaction.

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The Mast Cell System and Blood Coagulation in Mountain Dogs with Myocardial Infarction

V. I. Frolenko, G. A. Zakharov, G. I. Gorokhova, and N. P. Novikova

UDC 616.127-005.8-092.9-07:[616.155.36+616.151.5

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 6, pp. 580-583, June, 1994
Original article submitted October 11, 1993

A comparative study of the responses of the mast cell and coagulation systems to experimentally induced myocardial infarction in dogs that had been constantly living at a medium altitude (1600 m above sea level) and those constantly living at a low altitude (760 m) revealed less marked morphological and functional changes in these systems in the former ("mountain") dogs, which may be interpreted as an indication of their enhanced nonspecific resistance acquired as a result of long-term adaptation to conditions prevailing at medium altitudes.

Key Words: *myocardial infarction; mast cells; blood coagulation; adaptation; medium altitudes*

Studies in which the impact of various extremely adverse factors on the mast cell and coagulation systems was examined, have demonstrated that a relationship exists between the state of mast cells (MC) and alterations in the coagulation system [3,9-11]. This prompted us to investigate the state and role of MC in pathological conditions involving coagulation disorders, given that MC are known to be producers of heparin and other biologically active substances [4,14]. There is evidence that MC can ensure equilibrium of the coagulation-anticoagulation system, for each cubic millimeter of wall of a large human blood vessel contains as many as 1000 to 8000 MC [13]. Patients with myocardial infarction living in a plain region (around sea level) have been shown to have lowered blood levels of free heparin in conjunction with hypoplasia of MC and their reduced functional activity [7,8].

The objective of the present study was to examine the impact of myocardial infarction on the functional state of MC in relation to changes in

Laboratory for the Study of Respiration and Blood Circulation, Institute of Physiology and Experimental Pathology at High Altitudes, Academy of Sciences of the Kyrgyz Republic, Bishkek. (Presented by K. V. Sudakov, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0006-0579$12.50