tivating and its inhibitory action on ARC unit activity. However, the first mechanism evidently plays the decisive role.

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CORRELATION BETWEEN ACTIVITY OF THE KALLIKREIN, PLASMIN, AND THROMBIN SYSTEMS OF THE BLOOD DURING INTENSIVE PHYSICAL EXERTION

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Changes in the level of precursors of kallikrein, plasmin, and thrombin and of their inhibitors were studied by a combined method developed by the authors. The blood plasma of healthy athletes and of athletes with a syndrome of myocardial overstrain was investigated at rest and during intensive work on a bicycle ergometer. The results of the test and of correlation analysis reveal functional correlation between the components of the "Hageman factor system." In athletes with a syndrome of overstrain, the functional capacity of the humoral systems of vascular control was impaired, as reflected in a reduced level of proenzyme activity and a decrease in the values of the inhibitors. Correlation between the above-mentioned indices at rest and during exertion was disturbed in these subjects.

KEY WORDS: kallikrein–kinin system in man; physical exertion.

Adaptation of the hemodynamics to the factors of physical exertion takes place with the participation of the kallikrein system of the body. As a result of continuous physical training, the human kallikrein–kinin system undergoes modifications that reflect its increasing perfection as one of the regulatory mechanisms of the circulation [3, 4].

In the modern view, the kallikrein–kinin system of the blood is looked upon as being in functional unity with the fibrinolytic and clotting systems, as an important component of the mechanism of vascular control [1, 5, 7]. Changes in the activity of the fibrinolytic and clotting systems of the blood under the influence of physical exertion have been studied previously [6, 8].

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TABLE 1. Level of Precursors of Kallikrein, Plasmin, and Thrombin and of Their Inhibitors and of Protein in Blood Plasma of Athletes during Intensive Physical Exertion

Group of subjects and number	Time of investigation	Original arginine-esterase activity	Prekal-likrein	Kallikrein inhibitor	Plasminogen	Plasmin inhibitor	Prothrombin	Thrombin inhibitor	Protein, mg/ml plasma	Specific activity of total TAME-esterases
Blood donor (n=15)		2,4±0,5	62,8±2,9	0,91±0,09	88,3±4,5	0,05±0,02	81,0±4,6	1,53±0,07	—	—
Athletes:										
with syndrome of myocardial overstrain (n=10)	Before exertion	2,8±1,5	42,5±3,01*	0,67±0,09*	61,4±4,8*	0,21±0,12*	64,9±8,9	1,52±0,13	68,7±5,2	2,45
	After exertion	3,5±1,5	48,0±4,8	0,75±0,09	82,9±5,8**	0,43±0,14*	67,0±13,1	1,44±0,21	76,0±2,8	2,69
	Before exertion	8,9±2,5***	30,1±4,0***	0,61±0,10*	57,2±2,8*	0,12±0,05	74,5±9,01	1,52±0,07	73,5±4,2	2,31
	After exertion	4,1±1,1**	43,5±2,04**	0,62±0,07	70,8±3,6**	0,16±0,06	87,9±6,4	1,62±0,06	86,5±2,0**	2,33

Legend. 1) Activity of precursors expressed in micromoles TAME hydrolyzed per ml plasma per hour; concentration of inhibitors in conventional units.
2) Asterisks indicate significant changes ($P < 0.05$) compared with control (one asterisk), with state before exertion (two), and with healthy athletes at rest (three).

TABLE 2. Coefficients of Correlation (R) between Functionally Connected Pairs of Blood Systems in Healthy Athletes and Athletes with a Syndrome of Overstrain at Rest and during Work on a Bicycle Ergometer

	Healthy athletes (n=8)		Athletes with syndrome of overstrain (n=10)	
	rest	exertion	rest	exertion
Initial arginine-esterase activity (rest – exertion)	+0,97 †		—0,23	
Plasminogen (rest – exertion)	+0,87 †		+0,23	
Plasmin inhibitor (rest – exertion)	+1,00		+0,08	
Plasminogen – prekallikrein	+0,77*	+0,61*	+0,43	+0,56
Plasminogen – prothrombin	+0,57	+0,56	—0,15	—0,13

* Correlation significant at $P < 0.05$.
† Correlation significant at $P < 0.005$.

The investigation described below is a first attempt to study correlation between the activities of the three above-mentioned blood systems on the basis of a combined, unified method of determining the level of precursors of kallikrein, plasmin, and thrombin and of their inhibitors in athletes during intensive physical exertion.

EXPERIMENTAL METHOD

The investigation was carried out on 18 highly trained male athletes with a mean age of 22 ± 0.3 years. Eight of them were healthy, and in the other 10 a syndrome of chronic myocardial overstrain, due to continuous physical overloading (functional cardiopathy), was diagnosed. Blood samples were taken from the cubital vein of the two groups of subjects: the first sample at rest (the subjects rested beforehand in recumbency for 1 h); the second at the height of intensive physical exertion (work on a bicycle ergometer, in recumbency, with increasing power for 9-12 min). The criterion of the upper limit of loading was when the pulse rate reached 170-188 beats/min. At the beginning of work, the load was 450 kg·m, and every 3 min it was increased by 150 kg·m.

The content of precursors of kallikrein, plasmin, and thrombin and of their inhibitors in the blood plasma was determined by a combined method described previously [2]. The principle of the method is measurement of the TAME*-esterase activity of the above-mentioned enzymes after their separate specific activation in blood plasma. The protein concentration in the plasma was investigated by Lowry's method in parallel samples. Blood plasma from 15 donors, who were physically healthy and did not engage in sport, was used for the control tests.

* N-α-tosyl-L-arginine-methyl ester.

Statistical analysis of the results and of the indices of correlation between pairs was carried out by the usual method.

EXPERIMENTAL RESULTS AND DISCUSSION

The data given in Table 1 show that in the healthy athletes the prekallikrein concentration was reduced by 32.5% and the plasminogen concentration by 31% compared with the donors; there was a tendency for the concentration of kallikrein inhibitor and thrombin to fall. The level of plasmin inhibitor was increased fourfold. During physical training, not only the activity of the kallikrein–kinin system [4] but also that of other functionally connected systems of the blood plasma thus decreased appreciably.

In athletes with a syndrome of overstrain, the above indices differed even more from the control. At rest the prekallikrein concentration was reduced by 52%, the plasminogen concentration by 35%, and the concentration of kallikrein inhibitor by 33% ($P < 0.05$). The initial TAME-esterase activity of the plasma was increased by 2.4 times and, in conjunction with the lowered level of prekallikrein and kallikrein inhibitor, this must be regarded as evidence of imbalance of the kallikrein–kinin system. In athletes with a syndrome of overstrain the resting content of prekallikrein and plasminogen was lower than in healthy athletes.

During intensive physical exertion, in the form of a test of adaptive mobilization of the humoral systems of the body, changes indicating increased activity of the kallikrein and plasmin systems of the blood were observed. In healthy athletes changes in the components of the plasmin system were especially marked: The plasminogen level was raised by 34% and the level of plasmin inhibitor was doubled. This last index was thus 8.3 times higher than in the control. In athletes with a syndrome of overstrain the initial esterase activity was reduced by half during exertion, whereas the concentrations of prekallikrein and plasminogen were significantly higher than at rest (by 44 and 23.5%, respectively). A tendency was observed for the prothrombin level to rise (18%). In healthy athletes the response to exertion is thus linked chiefly with mobilization of the plasmin system. In athletes with a syndrome of overstrain, the increase in activity of the plasmin system was less marked, but by contrast the prekallikrein level was raised by a much greater degree. However, the changes in kallikrein and plasmin inhibitors in these athletes during exertion were small, a result which can be interpreted as weakening of the function of the systems controlling enzyme activity.

The results of determination of the plasma protein concentration showed an increase after exertion; these changes in the healthy athletes were no more than a tendency, whereas in athletes with a syndrome of overstrain they were significant ($P < 0.05$). The increase in the plasma protein level, coinciding with an increase in the concentration of precursors and inhibitors of the enzymes during loading, indicates rapid liberation of the components of the kallikrein and plasmin systems of the blood from their reserve forms. The specific activity of the total TAME-esterases in the plasma showed no significant change under these circumstances.

Additional evidence for assessment of the results was obtained by the use of statistical correlation analysis. Correlations were calculated between pairs of the principal indices for the athletes of both groups at rest and during exertion. By this method of analysis it was possible to test to what extent individual values of each index coincide with those of the group being compared, for all available cases.

Calculations (Table 2) indicate significant correlation between the indices of the initial arginine-esterase activity of the healthy athletes at rest and during exertion. In subjects with a syndrome of overstrain this correlation was disturbed. Determination of correlations between indices for plasminogen, plasmin inhibitor, and other precursors of the enzymes also point to weakening or disturbance of correlation between these indices if the athletes have a syndrome of overstrain both at rest and during intensive physical exertion. The combined simultaneous investigation of components of the kallikrein, plasmin, and thrombin systems thus yielded factual evidence of functional correlations between these systems. Attention is drawn to the plasmin system, which experienced the greatest changes, and which probably occupies a special place among the components of the "triad" of the Hageman factor systems.

Investigation of these important factors of vascular regulation also yields data relating to the syndrome of myocardial overstrain. Evidently in the athletes of this group, as a result of continuous physical overloading, the functional capacity of the humoral control systems is exhausted and this is reflected in low enzyme activity and depressed values of the inhibitors, followed by disturbance of interaction between them. These negative shifts are manifested particularly under conditions of intensive physical exertion.

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REFRACTORINESS OF RED BLOOD CELLS AND PLATELETS

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The effect of proteolytic enzymes and of arachidonic acid on aggregation of red blood cells and platelets was studied. These substances were found to stimulate aggregation of the blood cells. Preliminary incubation of fibrinolysin, trypsin, and arachidonic acid with suspensions of blood cells, however, is followed by a marked decrease in their ability to aggregate, i.e., by the development of a refractory state. The possible mechanism of this phenomenon is discussed.
KEY WORDS: blood cells; aggregation; refractoriness; proteolytic enzymes; arachidonic acid.

The refractoriness of platelets began to be studied only in the last decade [11, 13]. The essence of this phenomenon is that incubation of platelets with ADP causes them to lose their ability to react to fresh doses of ADP. The state of refractoriness lasts at least 4 h and, according to the authors cited, it is not connected with adenosine formation.

Although in recent years new investigations confirming the possible development of refractoriness of platelets during their incubation with ADP have been published [2, 3, 9], the mechanism of this phenomenon remains unexplained. As yet, moreover, the possibility of development of refractoriness of platelets has been described only in relation to ADP. The only exception has been the work of Eika [7], who showed that the second wave of adrenalin aggregation in heparinized plasma is depressed and collagen aggregation of the platelets is reduced; this is attributed to the development of a refractory state of the platelets. It is also not clear whether refractoriness is a specific property of platelets or whether other blood cells, in particular red blood cells (RBC), can also develop it.

The investigation described below was devoted to a study of these problems.

Arachidonic acid and the proteolytic enzymes fibrinolysin and trypsin were used as the aggregating agents. These substances were chosen for the following reasons. Increased proteolytic activity in the blood is a fairly frequent phenomenon and can take place in many different pathological states (shock, hypoxia, etc.) as well as during the treatment of thromboembolism by thrombolytic substances (plasmin, streptase, urokinase). Data showing the possible aggregating action of these enzymes on platelets have been published [10], whereas virtually no attempt has been made to study their effect on RBC. Arachidonic acid, which aggregates platelets, can be regarded in the opinion of several workers as the key factor of hemostasis and thrombosis [15]. The possibility of aggregation of RBC by arachidonic acid likewise has not been established.

EXPERIMENTAL METHOD

Aggregation of RBC and platelets was investigated photometrically in an aggregometer. The degree of aggregation was estimated by measuring the maximal amplitude of the aggregatogram (Ma). Donors' RBC (1 :400) and platelets (300,000-400,000/mm^3), washed and resuspended in physiological saline, were studied.

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