Urgent Reaction of the Complement System to Hypoxic Exposure in Rats Sensitive to Hypoxia

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Different modes of hypoxic exposure led to phasic changes in activities of the complement system components in rats sensitive to hypoxia starting from the first minutes of the posthypoxic period and persisting for 24 h and longer. The direction of shifts in the complement system depended on the duration and intensity of oxygen deficiency. Single one-hour interval hypoxia led to a moderate elevation of activities of virtually all the studied components. A more intense hypoxic exposure (1-h hypobaric hypoxia at a height of 5000 m) induced a biphasic response: reduction of activities of the majority of complement system components during the first hour of posthypoxic period and subsequent elevation of these activities above the normal. Exposure to severe hypobaric hypoxia (7000 m) led to a longer and more pronounced primary reduction of complement components activities, while the phase of their activity increase was blurred. Animal capacity to the formation of urgent tolerance of hypoxia was retained and increased with increasing the severity of hypoxic exposure. The complement consumption during the posthypoxic period was presumably a programmed reaction preventing hyperactivation of complement system components and essential for tolerance formation.

Key Words: *complement system components; hypoxia tolerance; posthypoxic period; hypobaric hypoxia; interval normobaric hypoxia*

The complement system is a part of the congenital immune system. Activation of the serum complement system is also essential for adaptive immune response. On the other hand, some data indicate that stress conditions of different kind, including those caused by recovery after acute oxygen deficiency (reperfusion syndrome), promote stimulation of the complement system, responsible for postreperfusion damage inflicted to tissues [6,8,9]. Uncontrolled intensification of this process can lead to the development of morbid conditions, autoimmune diseases, reduction of immunity, resistance, and viability. These states are characteristic of various cardiovascular diseases leading to deterioration of oxygen delivery to cells (for example, the reperfusion syndrome emerging during recovery of impaired blood supply during the postischemic period and accompanying numerous acute and chronic diseases). The usefulness of the complement system inhibition for reduction of tissue damage has been demonstrated not once [7,11-13].

However, the mechanism of complement activation under conditions of hypoxic exposure remains not quite clear. This question is principally important, because hypoxic therapy is widely used in modern medicine as a therapeutic modality and as a method for improving the nonspecific resistance and adaptation to stress. Despite the absence of experimental studies, there are good grounds to suggest that the complement system is directly involved in these processes, similarly as in reperfusion injury. The genetically determined

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phenotypical differences in the complement response to hypoxia have never been studied. On the other hand, these data are essential for the search for regulators and modulators of the complement system activity and development of approaches to their application in medicine for improving the defense characteristics of the immune system.

All this prompted this research. Functional activities of the first five components of the complement system (also called the complementogram [1]) are an important indicator of the complement system status. We therefore studied the dynamic characteristics of complementograms in rats sensitive to hypoxia, which were exposed to hypoxia according to different protocols: preconditioning (models promoting the formation of defense adaptive mechanisms) and initiating the development of stress reactions.

MATERIALS AND METHODS

The study was carried out on outbred rats sensitive to hypoxia (n=50). The dynamics of activity of complement components was studied on day 1 after single exposure to hypoxia in different modes.

In order to obtain a complementogram, sheep erythrocytes sensitized with rabbit antibodies are lyzed with rat serum titered in progressive double dilutions in the presence of the relevant reagent (donor serum free from the target component) for evaluation of C1, C1q, C2, C3, C4, C5 or without reagent for CH50 in a 96-well polystyrene micropanel. The number (T) of the half-lysis well observed visually without measuring devices of any kind is a convenient value for comparison of the complement component activity. This value can be easily conversed to titers (serum dilution causing half-lysis) $1:2^{T+1}$ or to the number of effective molecules of the component $3\times10^7\times2^{T+2}$ (number of holes in the erythrocytes, leading to their lysis, according to the one-hit mechanism) [2,3]. The initial activity was taken for 100% and activity in subsequent dilutions was calculated by comparing serum titers at which half lysis was observed.

Three models of hypoxia were used for evaluating the impact of duration and severity of hypoxic exposure for the time course of the studied components. Interval normobaric hypoxia (INH) and hypobaric hypoxia (HBH) in the preconditioning mode and acute HBH were used [4,5,10].

Interval hypoxia in the preconditioning mode is widely used in modern medicine as a therapeutic modality (hypoxic therapy) and for improving the nonspecific resistance. Our INH protocol consisted of 6-7 cycles of alternating hypoxic/atmospheric air respiration (10% O_2; 5 min and 21% O_2; 3 min). Hence, the net duration of hypoxic exposure was 30-35 min.

The preconditioning HBH (HBH-5000) consisted in "elevation" (in a pressure chamber) to an altitude of 5000 m (10% O_2) and 60-min exposure under these conditions. These training conditions were more rigid than in INH, presumably due to longer single hypoxic exposure (60 min).

Acute HBH (HBH-7000) was created by elevation in a pressure chamber to an altitude of 7000 m (4% O_2) and exposure for 60 min. This mode of hypoxic exposure is extremely severe. A one-hour exposure can lead to death.

Hence, using three models, it is possible to carry out hypoxic exposure of different duration (INH and HBH-5000) and severity (INH<HBH-5000<HBH-7000).

RESULTS

Activities of the complement system components increased in response to a single INH session. This increase was clearly seen as soon as 30 min after the hypoxic exposure and persisted for 24 h, reaching 125-230% (Table 1). The increase of activities was the most manifest for C3 and C4. For three of seven components (C1, C5, CH50) the changes were biphasic: elevation was preceded by a slight decrease in activities of the components during the first minute of the posthypoxic period. For two components (C5, CH50), the increase of activity following its reduction was minimum (≤125%).

TABLE 1. Time Course of Serum Complement Component Activities (% of Control) in Rats Sensitive to Hypoxia on Day 1 after a Single INH Session

Time after INH session	C1	C1q	C2	C3	C4	C5	CH50
Control	100	100	100	100	100	100	100
1 min	77	100	100	100	123	62	63
0.5 h	153	133	123	150	150	125	100
2 h	153	133	100	150	150	125	125
24 h	153	133	153	200	230	100	125

Exposure to medium severe hypoxia (HBH-5000) induced a biphasic response of the complement system components during the posthypoxic period. The initial reduction was followed by an increase in activities of the components. This reaction was observed for 6 of 7 components (Table 2). The reduction phase ensued as early as just 1 min after the end of hypoxic exposure and persisted for 2 h, reaching the maximum after 1 h. The exclusion was C4: its activity decreased only after 1 h and for just a short time. The reduction phase was followed by elevation of activities of all components, which was 130-503% after 24 h, *i.e.* more pronounced than after INH. High activities of the components persisted even after 72 h (Table 2). The increase was maximum for C2. Similarly as after INH, the greatest and longest reduction of complement activities and the least subsequent elevation were characteristic of C5 and CH50.

Severe hypoxic exposure (HBH-7000) also induced a biphasic response of the components during the posthypoxic period (Table 3). The reduction phase was characteristic of 6 of the 7 components. The exception was C4 characterized by a slight and stable (118%) elevation of activity. Activities of five of seven components decreased by 20-60% during the very first minute of the posthypoxic period and remained low for 2 h, this reduction being greater than in response to HBH-5000. The maximum reduction of activity, similarly as in previous experiments, was recorded for C5 (no subsequent elevation of activity) and CH50. Activity elevation phase ensued significantly later than after HBH-5000 in six of seven cases (only after 24 h). Activities of the complement components reached 118-168% of the initial level, *i.e.* lower than after HBH-5000.

Hence, activity reduction phase was prolonged after severe hypoxic exposure (HBH-7000) and its degree was greater. Activity elevation phase was therefore delayed significantly and its intensity was lower.

Hence, hypoxic exposure by different protocols led to changes in activities of the complement system components of different direction and degree in rats sensitive to hypoxia, manifesting from the first minutes of the posthypoxic period and persisting for 24 h and longer. The reaction of the complement system components to hypoxia in sensitive rats depended on the duration of exposure and degree of oxygen deficit (30 or 60 min; 4 or 10% O_2). These regularities were shown by analysis of the mean activities of the first 5 components of the complement system (Fig. 1). Changes in the complement components were minimum after single INH exposure (benign hypoxic model). Moderate elevation of activities of virtually all the studied components were recorded. A medium hypoxic exposure (HBH-5000) induced a biphasic response: reduction of activities of the majority of complement system components during

TABLE 2. Time Course of Serum Complement Component Activities (% of Control) in Rats Sensitive to Hypoxia throughout 3 Days after a Single Session of HBH-5000

Time after HBH-5000	C1	C1q	C2	C3	C4	C5	CH50
Control	100	100	100	100	100	100	100
1 min	62	71	89	80	125	80	60
0.5 h	79	80	100	63	100	63	80
1 h	62	100	63	40	63	40	16
2 h	125	100	126	80	125	80	64
24 h	250	200	503	160	250	160	128
72 h	125	200	126	160	250	160	128

TABLE 3. Time Course of Serum Complement Component Activities (% of Control) in Rats Sensitive to Hypoxia on Day 1 after a Single Session of HBH-7000

Time after HBH-7000	C1	C1q	C2	C3	C4	C5	CH50
Control	100	100	100	100	100	100	100
1 min	80	61	93	118	118	71	43
0.5 h	80	84	71	85	118	71	84
2 h	80	84	71	59	118	71	59
24 h	168	168	142	118	118	71	119

the first hour of the posthypoxic period was followed by their significant elevation above the normal level. The biphasic response was likewise induced by HBH-7000, but the duration and intensity of low activity phase increased, while the elevation phase was less pronounced or failed to develop. Activity reduction phase was absent or minimum in C4 in response to all three kinds of exposure, while the degree of its activity elevation was higher than that of other components. This value reduced in the following order: INH>HBH-5000>HBH-7000 (*i.e.* with intensification of hypoxic exposure). Hypoxic exposure of any kind inhibited activities of C5 and CH50 greater than of other components, the subsequent elevation of their activities being the minimum. This regularity increased in the following order: INH>HBH-5000>HBH-7000.

We think that the initial reduction of the functional activities of the complement system components after hypoxic exposure was caused by activation of the complement system and consumption of components as a result of this activation. It is difficult to say which factor, specifically, was responsible for activation of the complement during hypoxia. These activations could be different in hypoxic exposure of different mode and intensity or could differ quantitatively. The subsequent stage of recovery and even elevation of activity in comparison with the initial level was most likely caused by induced biosynthesis of the complement components by products of its activation. Induction of the complement synthesis by products of hypoxic exposure generated by other body systems (for example, by the stress component of hypoxia) is also probable.

The results suggest that stable elevation of the complement components activities without its previous reduction, manifesting in response to INH directly after hypoxic exposure, was an adequate justified response of the system to a weak and moderate stimulus. Low activity phase for the majority of the complement components during the initial period of posthypoxic recovery after exposure of higher intensity could result from their higher utilization. However, subsequent recovery and even elevation of activities of the majority of the components indicated that the capacity to their synthesis was retained. Prolongation of the period of low activities of the complement components and less pronounced subsequent increase of their activities after acute hypoxic exposure presumably reflected the complement system overstrain leading to regulatory imbalance between its synthesis and consumption.

All these phasic changes in activities of the complement system components, depending on the intensity of hypoxic exposure, developed in animals in parallel with the formation of urgent tolerance of hypoxia. We have previously shown that various modes

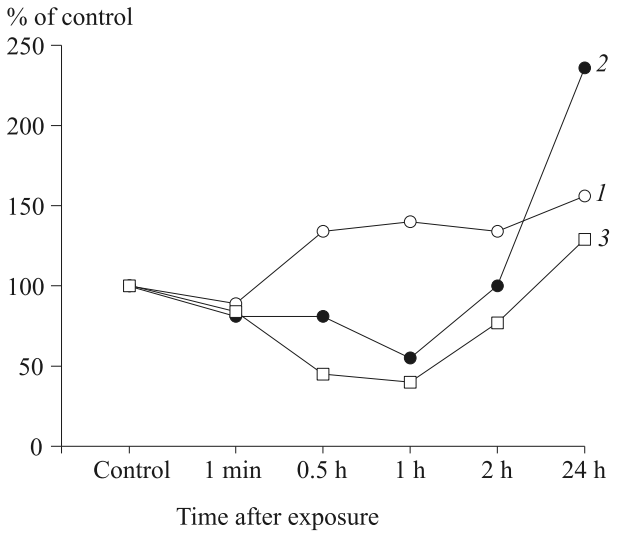

Fig. 1. Time course of summary activity of the first 5 complement system components (C1-C4) after hypoxic exposure of different mode in rats sensitive to hypoxia. *1*) INH; *2*) HBH-5000; *3*) HBH-7000.

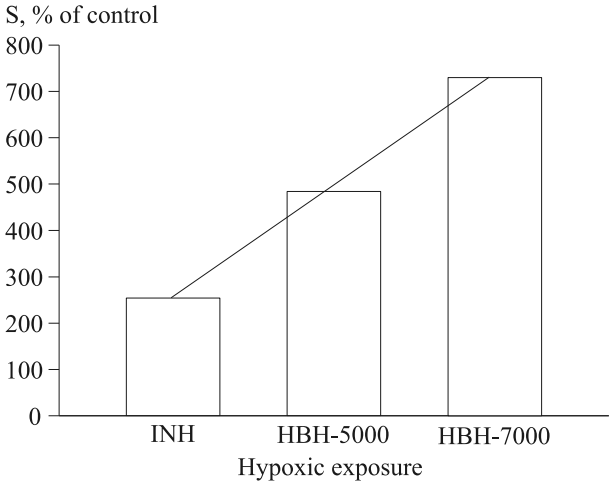

Fig. 2. Effect of a single hypoxic exposure on formation of urgent resistance in rats sensitive to hypoxia (S: survival at critical height incompatible with life).

of hypoxic preconditioning had different effects on the formation of urgent tolerance in rats [4,5,10]. The capacity of animals to tolerate subsequent exposure to severe hypoxia increased 2.5 times immediately after single 1-h INH (Fig. 2). After single HBH-5000 session, urgent resistance increased 5-fold, after HBH-7000 7-fold. Hence, the preconditioning effect increased in the INH>HBH-5000>HBH-7000 series, *i.e.* with increasing hypoxic exposure intensity, coinciding with increase of the low activity phase intensity for the majority of the complement system components.

On the whole, our data suggest that complement consumption during the posthypoxic period is a programmed body reaction essential for the formation of tolerance. Due to this, the total complement activity

decreased during the period preceding its rehabilitation at the expense of induced biosynthesis. The organism thus protects itself from damage caused by the complement stimulation and creates conditions for the formation of urgent mechanisms of adaptation. Subsequent elevation of the complement components activities as a result of their induced biosynthesis can be regarded as convalescence process.

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