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THE INCREASED CONCENTRATION OF 2,3-DIPHOSPHOGLYCERATE IN RED BLOOD CELLS OF SPONTANEOUSLY HYPERTENSIVE RATS

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It has been recognised that high haemoglobin oxygen capacity is essential for the development of high blood pressure in spontaneously hypertensive rats. In the present study we have found increased concentration of 2,3 diphosphoglycerate (2,3-DPG) in red blood cells of spontaneously hypertensive rats (SHR) of Okamoto-Aoki strain. As 2,3-DPG is the major factor decreasing haemoglobin affinity to oxygen, our finding suggests that at given value of pO_2 oxygen delivery to the tissue of SHR would be increased. Therefore increased concentration of 2,3-DPG in red blood cells of SHR would be of the pathophysiological meaning by promoting autoregulatory increase in total vascular resistance in this strain of rats. The mechanism responsible for enhanced synthesis of 2,3-DPG in SHR remains unclear. Intracellular alkalosis due to either hypocapnia and/or an enhanced activity of Na^+/H^+ antiporter occurring in SHR are the most plausible explanations for the above finding.

Key words: *2,3-diphosphoglycerate, spontaneously hypertensive rats, red blood cell.*

INTRODUCTION

The coexistence of a raised haematocrit and arterial hypertension in humans is widely recognised (1, 2). Erythorcytosis associated with hypertension was described as early as 1905 by Geisbock (3). Volhard and Fahr (4), in their classical textbook on arterial hypertension published in 1914, distinguished so-called red and pale hypertension.

Spontaneously hypertensive rats (SHR) of Okamoto-Aoki strain (5) are characterised by a high haematocrit (6). As plasma volume in SHR is not decreased, the raised haematocrit in this strain reflects an absolute increase in the red blood cell count in the circulating blood (7).

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Surprisingly little attention has been paid to clarifying the exact mechanism responsible for raised HCT both in humans with primary hypertension and in SHR (1, 2, 8). Similarly, the question regarding the pathogenic link between hypertension and raised HCT was until recently comparatively neglected. Over 20 years ago Sen *et al.* (6) showed the correlation between the increase in red blood cell count and values of systolic blood pressure during the natural history of the development of arterial hypertension in SHR.

It should be stressed that in arterial hypertension no factor leading to the secondary erythrocytosis-like, for instance, hypoxemia is known. It is well established that the level of 2,3-diphosphoglycerate (2,3-DPG) in red blood cells is decreased in primary erythrocitosis, whereas the opposite is true in the case of secondary forms of polycythemia.

In order to get more insight into the pathogenesis of erythrocytosis in SHR we evaluated the level of 2,3-DPG in arterial blood of SHR and their normotensive counterparts Wistar Kyoto rats (WKY).

MATERIALS AND METHODS

The experiments were carried out on 11 male SHR and 7 WKY at the age of 5 weeks; 9 SHR at the age of 14 weeks and on 7 age matched WKY. Animals, originally supplied from National Institute of Health, (Bethesda, Md., USA), were obtained from the animal house of the Department of Physiology, Warsaw Medical Academy. The rats were maintained three per cage on a 12-h light-dark cycle and fed standard labolatory chow and water *ad libitum*. Systolic blood pressure was measured by a tail photoelectric method in animals prewarmed for 10 min in the compartment in which the temperature was kept at 40°C. Three measurements on consecutive days were performed to allow animals to acclimatise to the new environment. On the next day after the final blood pressure measurement, animals were anaesthetized with pentobarbial sodium (Nembutal, Abbott, USA) at dose of 60 mg/kg. After limited laparotomy, the abdominal aorta was exposed and punctured with a thin needle. A capillary tube (100 µl.) was filled with arterial blood without contact with room air, and than 3 ml of blood was withdrawn by glass syringe. Animals were sacrificed by excess injection of pentobarbital sodium. The arterial gaseous composition and acid — base status was measured by an automatic blood gas system (AVL 995-Hb). Red blood cells counts (RBC) and their packed volumes (HCT) as well as haemoglobin concentrations (HGB) were measured by CELL-DYN 1600 (USA). Levels of 2,3-DPG in erythrocytes were determined using a Sigma Kit (ultraviolet detection, Sigma Chemical Company, St. Louis, Missouri, USA). Each sample was assayed in duplicate.

All values are presented as means ± SEM (standard error of mean). Student's t-test for unpaired data was used for statistical analysis; p. values less than 0.05 were considered as statistically different.

RESULTS

The values of systolic blood pressure in experimental and control groups are shown in *Fig. 1.* The values for HCT, RBC, HGB and 2,3-DPG levels in red blood cells did not differ between young and adult WKY. The values for RBC, HGB and 2,3-DPG are shown in *Fig 2, 3 and 4.* The highest values of 2,3-DPG were observed in 5 week old SHR.

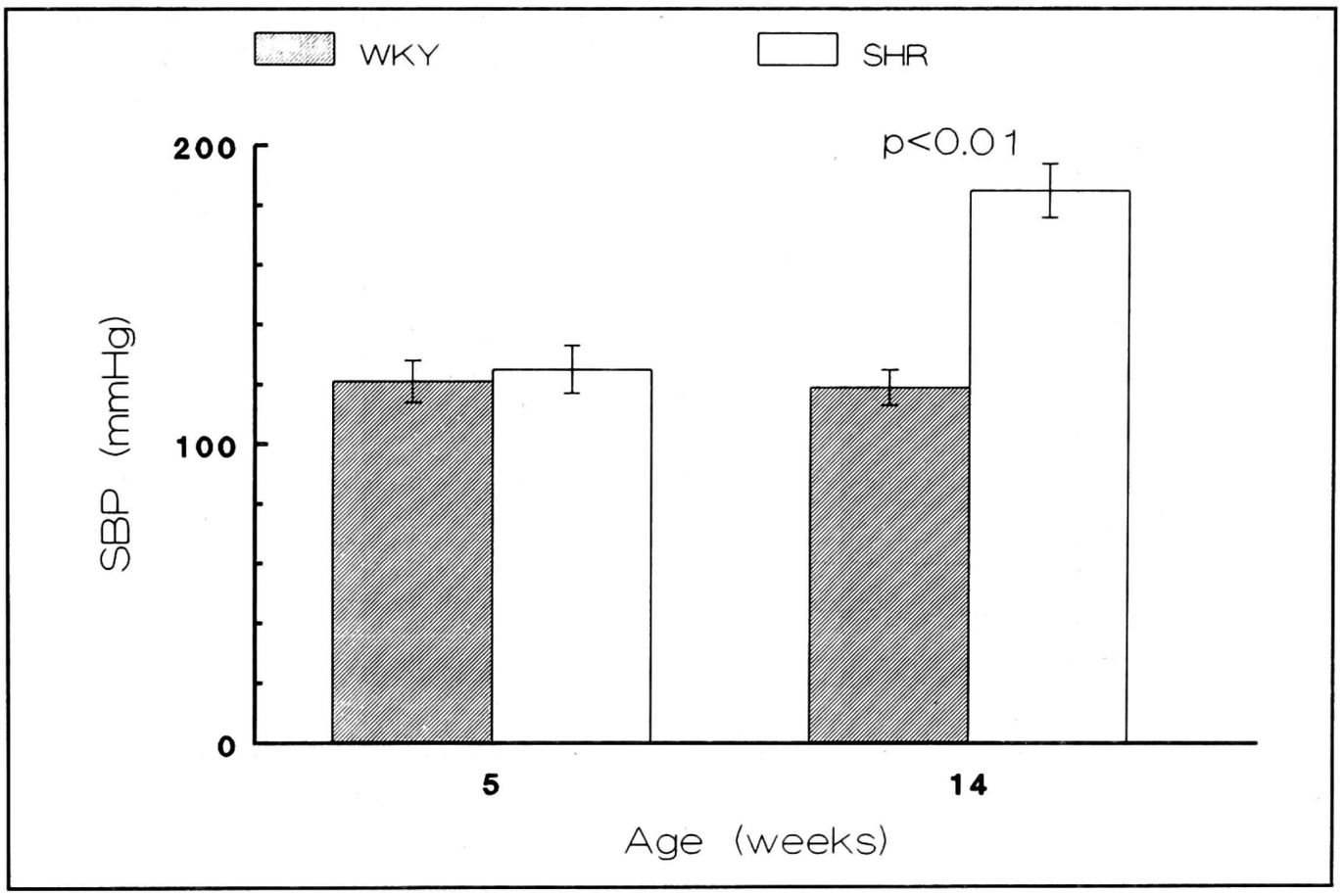

Fig. 1. Systolic blood pressure (SBP) in WKY and SHR rats at different ages (mean ± SEM, young SHR- n = 11, adult SHR- n = 9, young WKY- n = 7, adult WKY- n = 7).

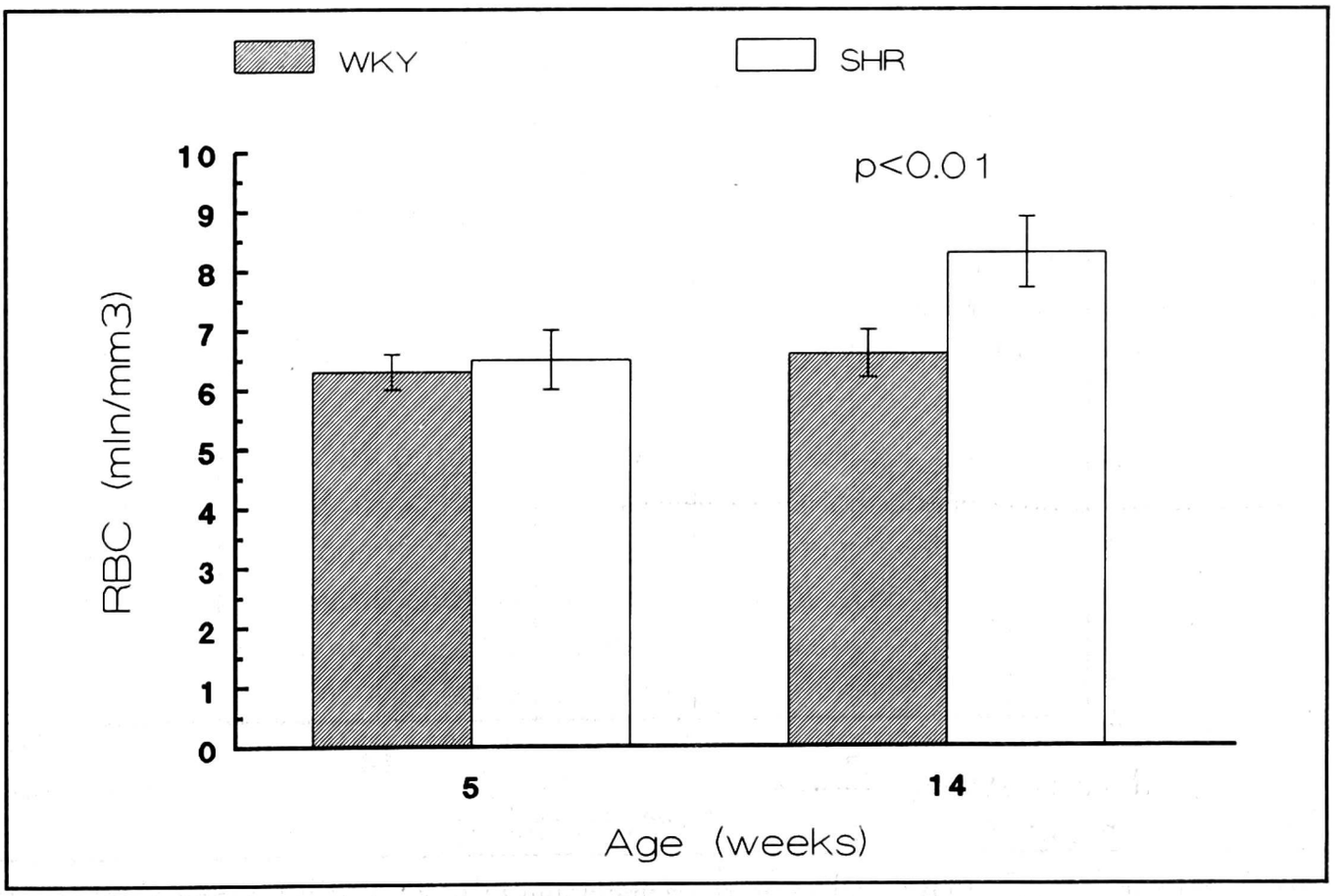

Fig. 2. Red blood cell count (RBC) values in WKY and SHR rats at different ages (mean ± SEM, young SHR- n = 11, adult SHR- n = 9, young WKY- n = 7, adult WKY- n = 7).

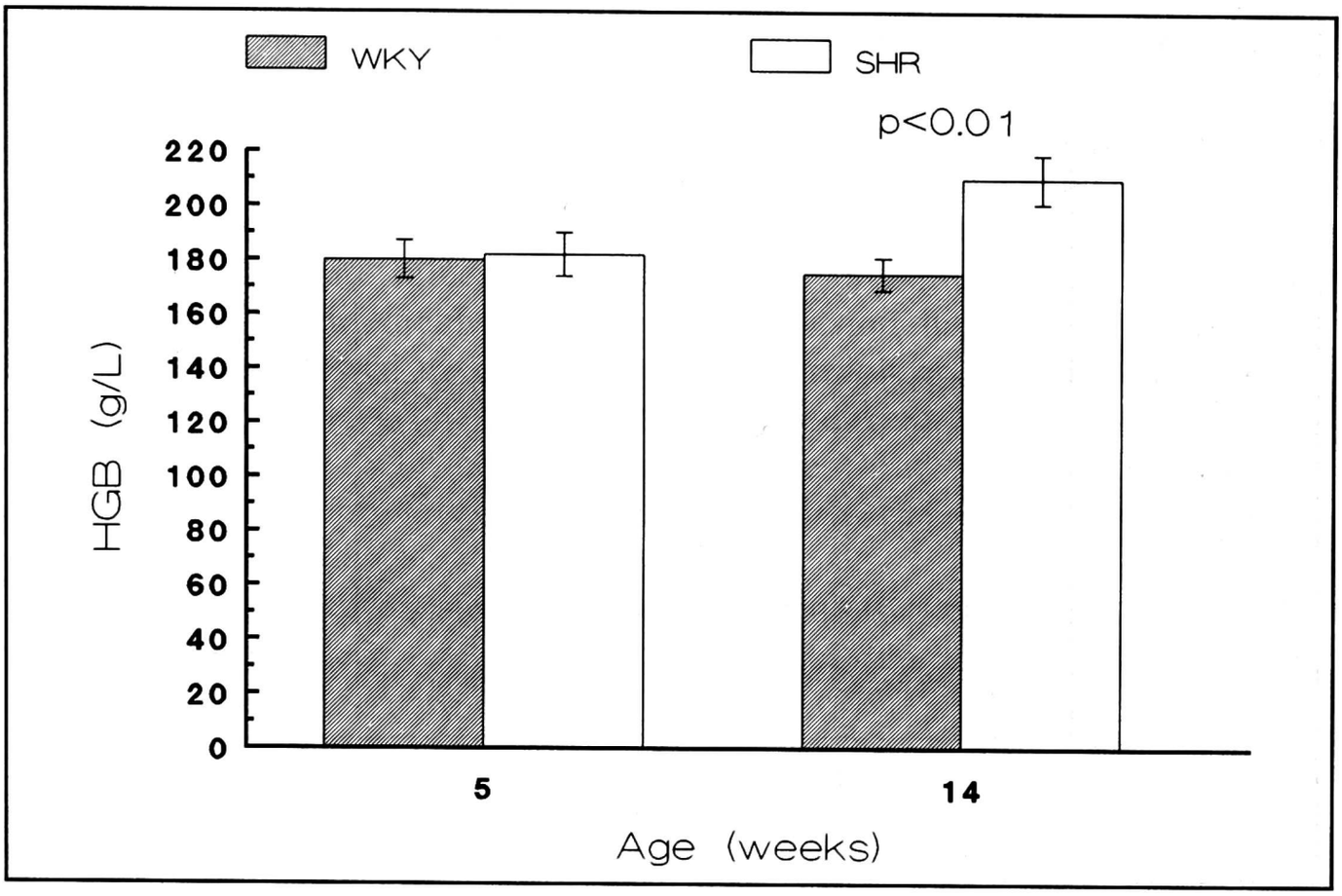

Fig. 3. Haemoglobin concentration (HGB) in WKY and SHR rats at different ages (mean ± SEM, young SHR- n = 11, adult SHR- n = 9, young WKY- n = 7, adult WKY- n = 7).

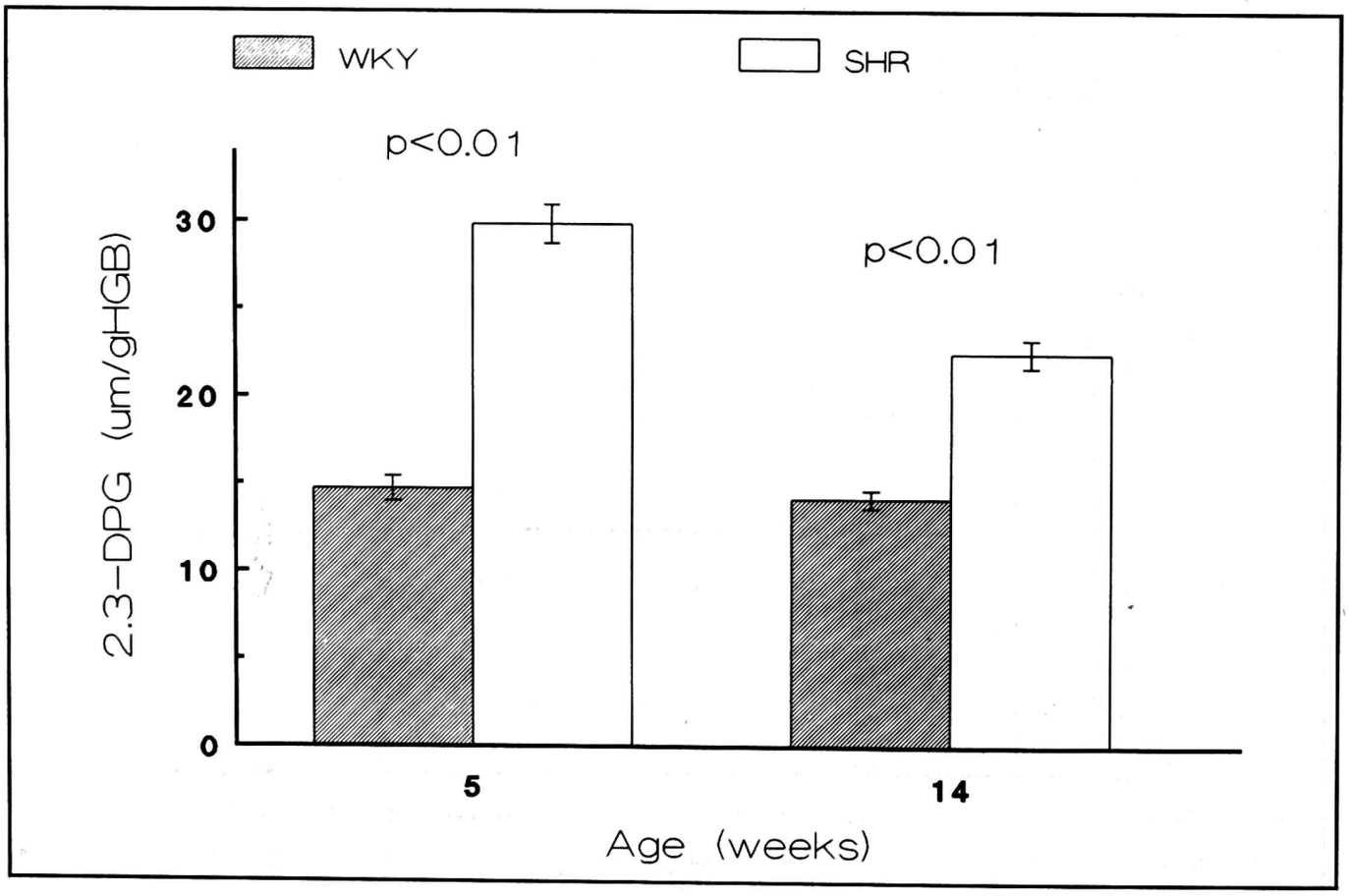

Fig. 4. Micromoles of 2,3-DPG per gram of haemoglobin (2,3-DPG) in WKY and SHR rats at different ages (mean ± SEM, young SHR- n = 11, adult SHR- n = 9, young WKY- n = 7, adult WKY- n = 7).

The level of 2,3-DPG in erythrocytes of 14 week old SHR was lower than in younger group, although still significantly higher than in control group (*Fig. 2*). The haemoglobin concentration, haematocrit value and red blood cells counts increased significantly only in adult SHR. Arterial pO_2, pCO_2 and pH did not differ among groups (*Table 1*).

Table 1. Arterial pO_2, pCO_2 and pH in WKY and SHR rats at different ages (mean ± SEM young SHR- n = 11, adult SHR- n = 9, young WKY n = 7, old WKY- n = 7)

Groups	WKY		SHR	
Age (weeks)	5	14	5	14
pO_2	86.6 ± 4.1	88.1 ± 3.5	90.1 ± 4.9	88.4 ± 4.2
pCO_2	39.4 ± 2.5	40.1 ± 2.9	36.7 ± 2.0	38.5 ± 1.6
pH	7.335 ± 0.03	7.343 ± 0.04	7.381 ± 0.04	7.372 ± 0.03

DISCUSSION

The high level of 2,3-DPG accompanying raised RBC values as well as haemoglobin concentration in adult SHR seems to suggest that erythrocytosis in this strain is not due to uncontrolled overproduction of red blood cells by bone marrow, as in the case of polycythaemia vera. The later condition is characterised by low concentration of 2,3-DPG in erythrocytes.

The second finding of the present study was that the increased content of 2,3-DPG in red blood cells precedes the occurrence of erythrocytosis and the development of the high blood pressure in SHR.

Experimental data have shown that chronic decreasing of RBC in SHR caused by repeated blood lettings or induction of haemolitic or nutritional anaemia prevented the development of high blood pressure in this strain of rats (9—11).

In the majority of publication (for review see ref. 7) the increased RBC frequently observed in arterial hypertension is perceived solely in terms of increased blood viscosity. However the role of increased blood visocity due to erythrocytosis in pathogenesis of hypertension seems to be overestimated. During the course of marked erythrocytosis caused by chronic hypoxemia arterial hypertension does not occur in normotensive rats (12). Moreover in SHR kept in simulated high altitude a marked decrease in blood pressure in spite of increased RBC was found (13). Finally in the recent study it has been shown that arterial hypertension caused by erythropoietin administration in the rat is not related to the increased RBC (14). In conclusion available experimental data allow us to exclude the increased blood visocity as a major link between increased RBC and arterial hypertension.

Any hypothesis dealing with the pathophysiology of essential hypertension has to take into account the following facts. Firstly, in the established phase of hypertension, the blood flow through all major vascular beds is unchanged (15). Secondly, a marked rarefaction of the microcirculation occurs parallel to the development of hypertension (16). In SHR a twofold decrease in the number as well as of the mean diameter of arterioles in cremaster muscle have been described (17). Similar changes have been observed in other tissues, including skeletal muscles and digestive system (13, 18). Therefore, decreased vascularity significantly contributes to high peripheral resistance in SHR (16).

Unchanged blood flow in the presence of microvascular rarefaction raises the question regarding the mechanism preventing tissue hypoxia. As oxygen demand is not decreased in SHR (15), the increased delivery of oxygen from the given volume of blood is the only explanation of the above phenomenon. Cleary, the increase level of 2,3 DPG due to the decreased haemoglobin affinity to oxygen may contribute to enhanced oxygen supply. Therefore it can be hypothesised that the increase in 2,3-DPG content in red blood cells of SHR is the result of tissue hypoxia resulting from the rarefaction of microcirculation.

Two facts, however, seem to refute the hypothesis that the increased 2,3-DPG is secondary event to tissue hypoxia. Firstly, microvascular rarefaction does not occur before the development of hypertension (19). Secondly pO_2 in mixed venous blood, reflecting whole body oxygenation is significantly increased in young SHR (20).

Alternatively, it might be assumed that an increase in 2,3-DPG, followed by an elevation in haemoglobin concentration, are primary events. These would lead to an excessive oxygen tissue supply. From this point of view changes in architecture of microcirculation can be seen as the expression of autoregulatory mechanism protecting tissue from local hyperoxia. In other words, rarefaction of the microcirculation in arterial hypertension would mirror an increase in vascularization during prolonged hypoxaemia (21, 22). The above hypothesis is strengthened by the finding that exposure of SHR to stimulated high altitude prevented both the development of arteriolar rarefaction and arterial hypertension (13). The hypotensive effect of experimentally-induced chronic anaemia in SHR (9, 10, 12) corresponds well with the above finding. In contrast to SHR, neither hypoxemia nor anaemia had influenced blood pressure in normotensive animals.

Walsh and Tobia (23) found that vascular resistance in the denervated vascular beds supplied by subclavian artery was significantly higher in young SHR than in their normotensive counterparts. As hypoxemia abolished the difference in vascular resistance between hypertensive and normotensive rats,

authors concluded that oxygen-dependent mechanism is responsible for the maintenance of elevated vascular resistance in SHR. Furthermore Lombard *et al.* (24) found that an increased pO_2 in an artifical tissue fluid suffusing cremaster muscle lead to more pronounced constriction and complete closure of arterioles in SHR than in WKY. The above findings point to the pivotal role of adequate oxygen delivery in the development and maintenance of arterial hypertension in SHR. In this respect a possible role of a decreased haemoglobin affinity to oxygen in SHR was not considered. Our finding of the increased concentration of 2,3-DPG in erythrocytes of SHR raises the question whether rightward shift of oxygen-haemoglobin curve contributes to the oxygen-dependent raise in vascular resistance in arterial hypertension.

Sen *et al.* (6) attributed the increased red blood cell count SHR to a higher concentration of erythropoietin. The mechanism responsible for the enhanced synthesis of 2,3-DPG in erythrocytes of SHR remains obscure and calls for investigation. As intracellular alkalosis stimulates the synthesis of 2,3-DPG (25, 26), the following findings might shed some light on the results of the present study. Firstly, in SHR, respiratory alkalosis due to overactivity of arterial chemoreceptors is already observed at the prehypertensive stage (20, 27, 28). In contrast to our previous studies (27) and those of others (29), in the present study respiratory alkalosis was not observed in young SHR. The most plausible explanation for this discrepancy is that in the present study animals were anaesthetised with pentobarbital sodium, which causes a marked depresion of respiratory drive (30). Interestingly, an enhaced activity of membrane Na^+/H^+ antiporter was found both in humans with primary hypertension and in SHR (31, 32). Therefore besides respiratory alkalosis an enchanced activity of Na^+/H^+ antiporter could be another important mechanism responsible for increased synthesis 2,3-DPG in erythrocytes of SHR.

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