DIFFERENCES IN RED CELL CONCENTRATION AND HEMATOCRIT READING DEPENDING ON VESSEL LOCATION AND BLOOD FLOW RATE

G. I. Mchedlishvili and M. N. Varazashvili

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KEY WORDS: red cell concentration in blood; local hematocrit reading; local blood flow rate; red cell distribution in vascular system; cerebral ischemia.

The red cell concentration in the blood and the related hematocrit reading are important parameters of the microcirculation, for they determine the viscous properties (flowability) of the blood and the oxygen supply to the tissues. Since last century the dominant view in physiology has been that the red cell concentration (hematocrit reading) in the blood, distributed between different organs, is generally speaking constant for a given organism. In that case, the place from which blood is taken and the state of the blood flow in that place ought not to have significant importance. More recently, however, experimental data have been obtained in physiology to show that the red cell concentration and associated hematocrit reading may not only differ in different parts of the vascular system, but also depend on the blood flow rate in them [1].

This paper gives new experimental data on differences in the red cell concentration and hematocrit reading in blood depending on the situation of the vessel in the circulation and the blood flow rate in it.

EXPERIMENTAL METHODS

Experiments were carried out on 33 adult rabbits of both sexes, anesthetized with urethane (1 g/kg, intravenously). The preliminary surgical operations included: isolation of one femoral artery to record the systemic arterial pressure (BP), and isolation of the femoral vein and its muscular branches in the contralateral limb in order to take blood samples from one of the muscular branches; a skin incision along the sagittal line in the neck for tracheotomy and isolation of the two common carotid arteries, beneath which ligatures were placed (to occlude them so as to create ischemia in the cerebral hemispheres), and trephining of the skull in the parietal region to measure the intensity of the cerebral blood flow and to take samples of blood from the pial veins. Samples were taken from a drop of blood, flowing freely after injury to the walls of the muscular and cerebral veins 1-1.5 mm in diameter by a needle, into a mixer to dilute it 1:200 times, and into the pipet of a hematocrit. Blood from the chambers of the heart was taken with a syringe by puncture of the chest wall between the ribs in the region of the apex beat. The red cell concentration in the blood was determined by the standard method in a Goryaev's chamber and the hematocrit reading was obtained by centrifugation of the blood under standard conditions: 3500g, average radius 5 cm, 10 min. The blood flow rate in the brain was determined by the hydrogen clearance method; the active platinum electrode, with surface area about 1 mm^2 was inserted into the cerebral cortex on the boundary between the frontal and parietal regions, close to the sagittal fibers, and the comparison electrode, consisting of a chlorided silver plate (1 cm^2) was sutured under the skin in the occipital region. Cerebral ischemia was induced by occlusion of both common carotid arteries by means of ligatures. The systemic BP was meassured with a Mingograf-81 electromanometer through a cannula inserted into the animal's femoral artery. Data on the red cell concentration in the blood and the hematocrit reading, which correlated closely, served in this case for cross-checking the results of determination. The numerical values were subjected to statistical analysis ($M \pm m$) and differences in each group of animals were determined by the difference method.

Laboratory of Physiology and Pathology of the Cerebral Circulation, I. S. Beritashvili Institute of Physiology, Academy of Sciences of the Georgian SSR, Tbilisi. (Presented by Academician of the Academy of Medical Sciences of the USSR M. M. Khananashvili.) Translated from Buyulletei' Eksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 387-388, October, 1986. Original article submitted February 14, 1986.

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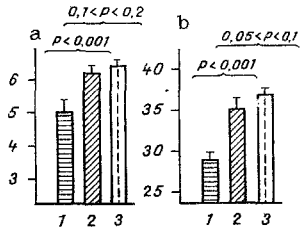

Fig. 1

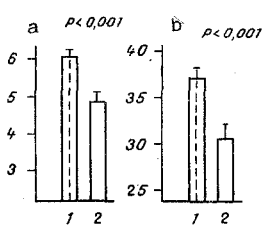

Fig. 2

Fig. 1. Differences in red cell concentration and hematocrit reading in blood samples taken from the heart (2) and also from veins of the hind limb (1) and brain (3) of rabbits. Ordinate: a) red cell concentration (in millions/mm^3); b) hematocrit reading (in %).

Fig. 2. Differences in red cell concentration and hematocrit reading in samples of venous blood taken from the brain, under control (1) and ischemic conditions (2). Legend as for Fig. 1.

EXPERIMENTAL RESULTS

In the animals of group 1 the red cell concentration and hematocrit reading in venous blood from the cerebral hemisphere was higher in all 22 rabbits (on average by 25 and 20% respectively) than in blood from the hind limb ($P < 0.001$). This confirms our previous findings [2] and is evidence that the red cell concentration and hematocrit reading are not the same in blood circulating in different organs. The fact that the distribution of red cells in the blood favors the brain means that its structural elements, which have high metabolic activity, receive a better oxygen supply.

In group 2, consisting of nine rabbits, the red cell concentration and hematocrit value in blood samples taken from the femoral and cerebral veins were compared with those in blood samples from the heart. Blood from the heart was found to have a higher red cell concentration and hematocrit reading in all the animals (by 20 and 17% respectively) than blood from the hind limbs. However, the differences between parameters for blood taken from the heart and blood taken from the cerebral vein were less marked: on average by 3 and 5% respectively (Fig. 1). In 49% of cases blood taken from the heart contained rather fewer red cells and had a lower hematocrit reading than blood from the brain; in 30% of the cases these parameters were a little higher and in 11% they were virtually identical with each other. The differences in this case were not significant: $0.1 < P < 0.2$ for the red cell concentration and $0.05 < P < 0.01$ for the hematocrit reading. This inconstancy of the results could be due to the superior technique used in taking blood from the heart.

Consequently, blood pumped from the heart into the arterial system of the systemic circulation arrives in different parts of the body containing different numbers of red cells and with different hematocrit values as a result of an unequal distribution of red cells and plasma along the length of the arterial branches, starting from the arch of the aorta; in the brain, moreover, these parameters are significantly higher in value than in the caudal part of the body.

In the 11 rabbits of group 3, in which blood samples taken from the pial veins in the control (untreated) and animals with ischemia showed that with slowing of the blood flow on average from 135 to 69 ml/g/min, i.e., by 51% (under these circumstances because of occlusion of the carotid arteries, blood entered the cerebral hemispheres only along collateral channels), the red cell concentration and hematocrit reading in the blood were considerably reduced: by 21 and 17.4% respectively (Fig. 2). This reducing in the red cell concentration in an ischemic organ is in agreement with data obtained previously in experiments on the retrolingual membrane of the frog [3].

A reduction in the red cell concentration in the blood during ischemia may have the result that the tissue hypoxia, caused by slowing of the blood flow, is aggravated still further

by a reduction in the number of red cells in the blood flowing along the microcirculatory bed of the organ. However, a reduction in the number of red cells in the blood flowing along the microvessels under these conditions may, at the same time, perhaps improve its rheologic properties, increase its flowability and thus play a compensatory role in ischemia [4].

Consequently, important hematologic parameters such as the red cell concentration in the blood and the hematocrit reading may vary considerably in the vascular system depending both on the place from which the blood samples are taken and the local blood flow rate.

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ROLE OF CALMODULIN IN MYOCARDIAL CONTRACTILITY

B. I. Laptev, S. A. Afans'ev,
V. D. Prokop'eva, L. V. Antoshkin,
and N. P. Larionov

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Calmodulin is an activator of many enzymes, including those of the Ca^{2+}-pumps of the sarcolemma [5] and sarcoplasmic reticulum [6] – systems responsible for regulating the intracellular Ca^{2+} concentration. Drugs of the phenothiazine series, which can inactivate calmodulin, are often used in investigations of calmodulin-dependent processes. In the study of cardiac contractility, only one or two preparations with closely similar affinity for calmodulin are usually used [2, 4, 10]. Recently published research has shown that drugs of the phenothiazine series can not only bind with calmodulin and, correspondingly, inactive Ca^{2+}-calmodulin-dependent enzyme systems, but can also interact independently of calmodulin with Ca^{2+}-binding proteins [1, 8], and α-adrenoreceptors [9], and they may also have a membrane-stabilizing action [7]. In our opinion, in order to interpret more correctly the causes of the changes in contractility parameters when phenothiazones are used, the effect of several drugs of this group, with different affinity for calmodulin, ought to be compared.

The aim of this invetigation was to compare the action of compounds of the phenothiazine series, namely trifluoperazine (TF), frenolon (PR), majeptil (MJ), and chlorpromazine (CP), on contractility of the rat papillary muscle.

EXPERIMENTAL METHODS

Experiments were carried out on papillary muscles isolated from the left ventricle of male Wistar rats, contracting under isometric conditions at 31°C. To reduce the effect of the phenothiazine on α-adrenoreceptors, prazosin (10^{-7} M) was added to the perfusion solution 20 min before they were injected. There were five series of experiments: series I was the control (seven experiments); in series II, III, IV, and V the following substances were added respectively to the perfusion solution; TF (eight experiments), FR (seven experiments), MJ (seven experiments), and CP (six experiments). The Ca^{2+} concentration in the perfusion solution was increased from 2 to 4 mM (the Ca^{2+} test) 20 min after addition of the test preparation (10^{-5} M in all cases), but the frequency of stimulation before and during the Ca^{2+} test was temporarily increased from 0.5 to 2 Hz. The force of contraction and its first

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Laboratory of Clinical and Experimental Physiology and Laboratory of Metabolism of the Heart, Siberian Branch, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Tomsk.

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