REDISTRIBUTION OF ERYTHROCYTES IN RAT MESENTERIC MICROVESSELS DURING REPERFUSION AFTER SHORT-TERM ISCHEMIA

V. K. Khugaeva

UDC 616.381-005.4-008.66-07:
616.155.1-031:611.16

KEY WORDS: ischemia; microvessels; hematocrit; leukocytes.

It has been suggested that the principal mechanism of the "no reflow" phenomenon is edema of the endothelium of the microvessels [5, 10, 12]. Clinical studies of ischemic heart disease and of arterial occlusion of varied etiology have revealed evidence of a disturbance of the rheologic properties of the blood *in vitro* [3, 4, 14]. Meanwhile only a limited number of papers have been published on the intravital study of the rheologic properties of blood [2, 6, 7, 9, 11, 15], and only single analogous investigations under conditions of ischemia [8]. In recent years information has been obtained on the important role of blood cells and, in particular, of leukocytes, in the pathogenesis of the "no reflow" phenomenon [13].

The aim of this investigation was an intervital study of the dynamics of the blood microcirculation and of the rheologic properties of blood (the hematocrit index — HI) in the mesenteric microvessels during perfusion after short-term ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 26 non-inbred male albino rats weighing 180-320 g, anesthetized by intramuscular injection of urethane (1.6 g/kg). Ischemia of a limited region of the mesentery of the rat small intestime was induced under the biomicroscope by compression of an afferent arteriole 30-60 μ in diameter by means of a miniature glass rod, fixed to a micromanipulator. Intravital determination of HI in branches of the afferent arteriole was carried out by flash photography [1]. HI was studied in 204 microvessels: 40 metarterioles 11-16 μ in diameter, 122 vessels of capillary type (pre- and postcapillaries, capillaries) 6-10 μ in diameter, and in 42 arteriolovenular anastomoses (AVA) 8-12 μ in diameter. HI was studied in each microvessel before ischemia and during 40 min of reperfusion. Each value of HI in a single microvessel was the average of 3 to 5 determinations. Repeated determination of the momentary value of HI in the microvessels was necessary because of physiological fluctuations in its value depending on vasomotor activity of the microvessels, the phase of cardiac contraction (systole or diastole), changes in the blood flow rate, the periodic pattern

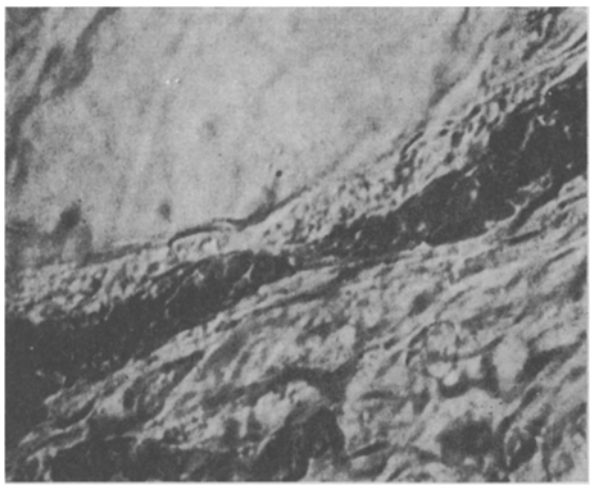

Fig. 1. Narrowing of lumen of arteriole by juxtamural thrombus formed after compression of vessel for 1 min. Biomicroscopy. 210×.

Scientific-Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. (presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 11, pp. 539-542, November, 1986. Original article submitted March 6, 1986.

 0007-4888/86/0011-1510$12.50

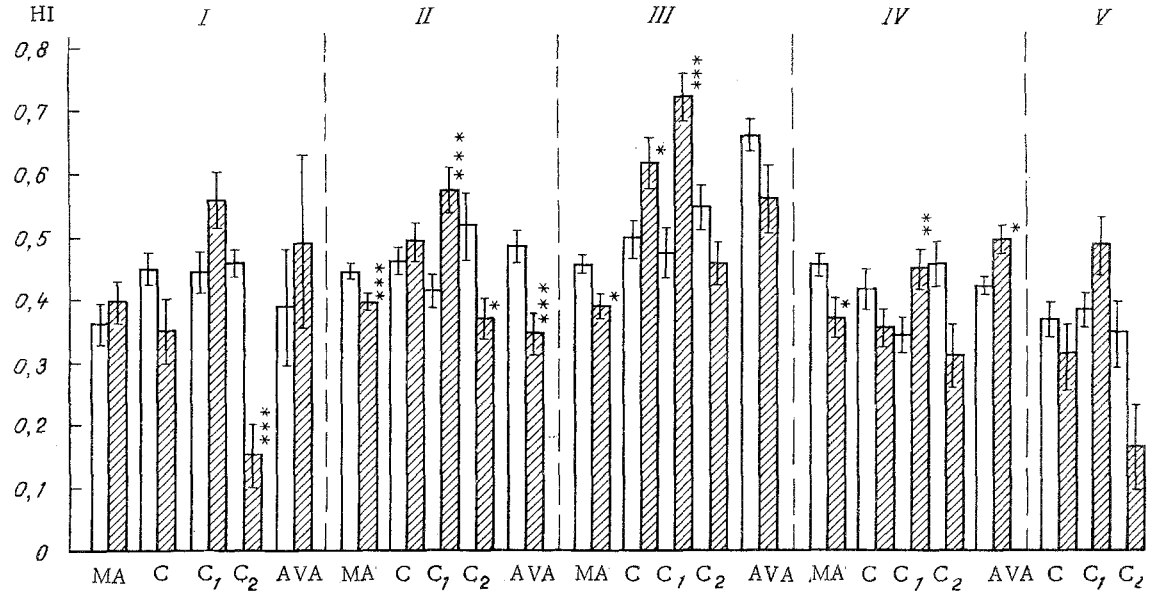

Fig. 2. Changes in HI (M ± m) in rat mesenteric microvessels during reperfusion after ischemia for 1 min. Periods of reperfusion: I) 1-2 min; II) 3-10 min; III) 11-20 min; IV) 21-30 min; V) 31-40 min after ischemia lasting 1 min. MA) Metarteriole; C) capillaries. Unshaded columns, initial value of HI before ischemia, shaded columns, HI during reperfusion. *P < 0.05; **P < 0.01, ***P < 0.001: levels of significance of change in HI during reperfusion compared with initial value.

of functioning of individual capillaries, etc. Heparin was applied to the surface of the mesenteric microvessels in a dose of 30-50 UI in 0.1 ml of isotonic sodium chloride solution. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Mechanical compression of the afferent arteriole for 1 min caused complete cessation of the blood flow in the compressed segment of the arteriole. In the postischemic period the blood flow was restored in only 76% of compressed arterioles, and in the other 24% of arterioles the blood flow was not restored because of thrombus formation in the compressed segment (Fig. 1). Recompression of the vessel or lengthening of the duration of compression of the afferent arteriole to 3-5 min was accompanied by an increase in the frequency of thrombus formation to 50% (in 8 of 16 vessels). The probability of thrombosis in the segment of compression increased with a decrease in diameter of the compressed vessel.

Thrombosis of the afferent mesenteric arteriole caused total cessation of the blood flow in its peripheral branches only if the network was poorly developed, when a characteristic feature was the absence of proximal anastomoses and communications with other vascular networks. Most mesenteric afferent arterioles (diameter 30-60 μ) had a branching network of microvessels, rich in anastomoses with other arterioles and branches. Thrombosis of such an arteriole did not cause complete cessation of the blood flow in its branches, but simply reduced it. The reduced inflow of blood into branches of the thrombosed arteriole was maintained initially by the inflow of blood via AVA. After the AVA had ceased to function the blood flow was maintained in the retrograde direction: through peripheral branches of an arteriole connected with other vascular branches, in central segments of the arteriole, and from thence into its branches. Retrograde inflow of blood from venules was possible for capillaries. However, the additional three ways of inflow of blood into the ischemic region of the mesentery did not succeed in restoring the blood flow completely or for a long time in the peripheral branches of the thrombosed arteriole. In the course of reperfusion of this kind for 17-20 min stasis developed in the microvessels. Postischemic disturbances of the blood flow, together with other local factors, led to thrombosis of the main afferent trunk and inadequate compensation of the deficient blood flow into the ischemic region along the accessory inflow pathways.

In most afferent arterioles (76%) the blood flow was restored at once after compression lasting 1 min was removed. Particular attention was paid to this group of microvessels, be-

cause the cause of the postischemic disturbances of the microcirculation in them is not clear.

Short-term (1 min) ischemia of the branching microvascular network of the mesentery, due to interruption of the inflow along the afferent arteriole, was accompanied by reduction of the inflow of blood and of HI in the whole of the microvascular network. In metarterioles HI was reduced by 21%, in capillaries by 33%, and in AVA the greatest decrease in HI (46%) was observed, compared with its initial value (before the beginning of ischemia). Restoration of the blood flow along the afferent arteriole after the ending of its compression was accompanied by rapid restoration of HI in metarterioles and in AVA (Fig. 2, I). During the first 2 min of reperfusion (period I) HI in the metarterioles and AVA was higher in absolute terms than its value before the beginning of ischemia, evidence of reactive hyperemia of the microvessels in response to the termination of ischemia. Recovery of HI had not yet taken place in the capillaries, and its value was still below the initial level. However, a detailed study of HI in the capillaries showed that they could be divided into two groups: C_1 and C_2, depending on the time course of HI. In the C_1 group, numbering 50% of all capillaries, a very small rise of HI was observed, whereas in the other capillaries (C_2) HI was sharply reduced, and for that reason the overall response of the capillaries was characterized by a reduction of HI. In the next 3-10 min of reperfusion (period II) the value of HI decreased in the metarterioles and, in particular, in AVA. The overall response of the capillaries was characterized by restoration of their initial value of HI. The distribution of HI in the capillaries was as follows: In 62% (C_1) it was increased, in 38% (C_2) it was reduced (Fig. 2, II). Since the degree of increase in HI in the C_1 group and of its decrease in the C_2 group was similar, the overall response was the same as initially. In period III of reperfusion (11-20 min) the low level of HI was preserved in the metarterioles and AVA, but it began to increase in 62% of capillaries (Fig. 2, III). The number of capillaries with a low HI fell to 38%. The degree of lowering of HI in the C_2 group was less than the degree of its increase in the C_1 group, and for that reason the overall response of the capillaries was characterized by an increase in HI. The question may reasonably be asked: why during periods II and III of reperfusion (18 min), despite a decrease in the inflow of erythrocytes into the metarterioles and AVA, the number of erythrocytes in the capillary bed progressively increased. This redistribution of erythrocytes in the capillary bed is possible only if their outflow into the venular bed is obstructed. In periods II and III of reperfusion, biomicroscopy revealed a marked increase in pavementing of the leukocytes in the venules and postcapillaries. Slowing of the blood flow could be observed visually. Concentrations of aggregated leukocytes formed juxtamural thrombi, which narrowed the lumen of the microvessels and disturbed the laminar nature of the blood flow. Application of heparin (30-50 UI) to the surface of the microvessels did not affect adhesion of the leukocytes to the inner surface of the microvascular wall and did not restore the normal blood flow. In period IV of reperfusion (21-30 min) HI remained low in the metarterioles, but by contrast with the previous periods, it was increased for the first time in AVA (Fig. 2, IV) against the background of progressive slowing of the blood flow in the microvessels. Consequently, the increase in HI in AVA is evidence, not of an increased blood flow, but of slowing of the retrograde inflow of blood into the capillaries. The latter become emptied: In 67% of capillaries a decrease in HI was observed, due to reduction of the inflow of erythrocytes along the main path of inflow through metarterioles and to retention of erythrocytes in AVA. The redistribution of erythrocytes and HI in the microvascular network described above preceded the development of stasis in the next period V (31-40 min), which developed first of all in AVA and the metarterioles. The slowed blood flow in the capillaries was maintained for a short time (1-5 min) by retrograde inflow of erythrocytes from the venular bed. During this period HI fell considerably (Fig. 2, V) in most capillaries (62%, C_2) and was a little higher than initially only in 28%. Thus the mesenteric capillary bed in the postischemic period after ischemia lasting 1 min was the most resistant and was the last to become excluded from the blood flow and not the first, as might be supposed.

Measurement of the diameter of all the microvessels studied before and during reperfusion revealed no significant changes in the lumen of the microvessels. More rapid (after 17-20 min) disturbances of the microcirculation after ischemia lasting 1 min occurred in the case of thrombosis of the afferent arterioles, and were due to reduction of inflow into the microcirculatory bed. In the absence of thrombosis the blood flow in the vascular network also was disturbed, but later (after 30-40 min). An important role in this disturbance of blood flow in the microvessels is played by the abrupt increase of adhesion of the leukocytes to the microvascular wall. Total "no reflow" in the mesentery in the postischemic period is ob-

served rarely, and only in undeveloped vascular networks. In most vascular networks there is a gradual disturbance of the blood flow, depending on the duration of reperfusion.

LITERATURE CITED

1. P. N. Aleksandrov, Current Problems in Disease and Recovery [in Russian], Moscow (1981), pp. 71-74.
2. V. I. Kozlov and E. K. Kistanova, Fiziol. Zh. SSSR, No. 11, 1527 (1984).
3. V. A. Lyusov and M. P. Savenkov, The System Regulating the Aggregate State of the Blood under Normal and Pathological Conditions [in Russian], Moscow (1982), pp. 48-59.
4. V. V. Progonnaya and A. I. Romanenko, Vrach. Delo, No. 4, 39 (1982).
5. L. I. Ramazanova and E. A. Bardakhch'yan, Kardiologiya, No. 2, 82 (1985).
6. V. K. Khugaeva and P. N. Aleksandrov, Byull. Éksp. Biol. Med., No. 12, 17 (1982).
7. B. Fagrell, V. Ostergen, and M. Intaglietta, Microvasc. Res., 17, No. 3, Part 2, 545 (1979).
8. G. Kanzow, A. R. Pries, and P. Gaethgens, Recent Advances in Microcirculatory Research, Basel (1981), pp. 149-152.
9. P. Klitzman and P. C. Johnson, Recent Advances in Microcirculatory Research, Basel (1981), pp. 144-148.
10. B. Lee, S. Wilson, R. Domenech, and D. MacGregor, Can. J. Surg. Res., 29, 50 (1980).
11. H. H. Lipowsky, S. Stemmer, S. Usami, and S. Chien, Microcirculat. Clin, Exp., 3, 301 (1984).
12. G. Rona, M. C. Badonnel, I. Hütiner, et al., Exp. Mol. Pathol., 31, 211 (1979).
13. G. W. Schmid-Schönbein and R. L. Engler, Microcirculat. Clin. Exp., 3, 336 (1984).
14. V. R. Sternitzky and K. Seige, Z. Ges. Inn. Med., 38, 1 (1983).
15. G. J. Tangelder, H. C. Teirlinck, and D. W. Slaaf, Am. J. Physiol., 248, No. 3, H318 (1985).

Na^+-K^+ COTRANSPORT IN THE ERYTHROCYTE MEMBRANE IN PATIENTS WITH ESSENTIAL AND SYMPTOMATIC (RENAL) HYPERTENSION

V. G. Kavtaradze, N. G. Aleksidze,
N. K. Kvirikadze, and N. I. Nadiradze

UDC 616.12-008.331.1-07:
616.155.1-008.923.2/.3

KEY WORDS: hypertension; erythrocytes; Na^+ and K^+ cotransport; diagnosis.

Changes in permeability of erythrocyte membranes for Na^+ and K^+ ions have been observed in patients with essential hypertension and in its experimental model, namely, spontaneous hypertension in rats. Changes in permeability of erythrocyte membranes also have been discovered in persons with normal blood pressure but whose parents are hypertensive [2, 7]. Changes in this kind are not found in symptomatic arterial hypertension, whether experimentally or clinically [8, 9].

The aim of this investigation was to study changes in permeability of erythrocyte membranes for Na^+ and K^+, with particular reference to Na^+-K^+ cotransport in erythrocytes of patients with essential hypertension and with sympotomatic (renal) hypertension, taking hereditary factors into account.

EXPERIMENTAL METHOD

Erythrocyte permeability was studied in 38 patients with stage I and II of essential hypertension (WHO classification; 12 women and 26 men aged from 30 to 58 years), in 12 patients with symptomatic (renal) hypertension (seven women and five men aged from 40 to 59 years), in nine patients (two women and seven men aged from 26 to 54 years) in whom a raised blood pressure (BP) up to the higher limit of normal was observed episodically (borderline hypertension), and in 15 persons (three women and 12 men aged from 34 to 49 years)

Academician M. D. Tsinamdzgrishvili Scientific-Research Institute of Clinical and Experimental Cardiology, Ministry of Health of the Georgian SSR, Tbilisi.

(Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 11, pp. 542-543, November, 1986. Original article submitted February 18, 1986.