MICROVASCULAR WALL THICKNESS AND REACTIVITY TO VASOCONSTRICTOR STIMULI IN SKELETAL MUSCLES OF NORMAL AND SPONTANEOUSLY HYPERTENSIVE RATS

M. I. Timkina and O. V. Dolgun

UDC 616.12-008.331.1-07:/616.16-031:611.73/-091-02:615.225.1

KEY WORDS: spontaneously hypertensive rats; microcirculation of skeletal muscles; vascular reactivity; hemorrhage.

Spontaneous hypertension in rats, which we chose as the model of persistent elevation of the systemic arterial pressure (BP) and the pressure in the microvessels [4], has been intensively studied from the standpoint of vascular reactivity. The majority of experimental studies in this direction, which have used a wide range of techniques and objects, have yielded evidence of increased reactivity of the blood vessels of spontaneously hypertensive rats (SHR) to vasoconstrictor stimuli compared with normotensive rats (NR) [7, 12]. The structural basis for the increase in reactivity of the vessels and in the strength of their contraction in SHR is considered to be a marked increase (up to 40%) in thickness of the vascular wall and a decrease in the lumen of the vessels, which also has been found in the microvascular bed of sleketal muscles [5, 8].

The aim of this investigation was to compare the absolute and relative thickness of the vascular wall and the degree of contraction of arterioles in skeletal muscles of SHR and NR of the Wister strain in response to stimulation of the sympathetic chain.

EXPERIMENTAL METHOD

Experiments were carried out on 12 NR weighing 230-250 g and 10 SHR aged 16-18 weeks and weighing 210-280 g. The animals were prepared for the experiments and biomicroscopy of the extensor hallucis proprius (EHP) and stimulation of the sympathetic chain were carried out as described previously [3], the only difference being in the parameters of stimulation: the strength of current was 6 mA, pulse frequency 3 Hz and duration 0.5 msec, and period of stimulation 10 sec. After adaptation of the preparation for 30 min the external diameter and diameter of the lumen of the microvessels were measured under the microscope with magnification of 58, 164, and 250, after which the thickness of the wall could be determined and its ratio to the radius of the lumen (w/r) calculated. The sympathetic chain was stimulated 3 to 7 times in each animal with an interval of not less than 5 min, and during maximal vasoconstriction, the same parameters were again measured. To assess reactivity of the microvessles, which in the present context is equivalent to the effectiveness of vasoconstrictor influences ($\Delta D\%$), the formula $(D_0 - D)D_0 \times 100\%$ was used, where D_0 is the diameter of the lumen before stimulation of the chain and D its diameter during the maximal contractile response to a given standard stimulus. Acute hemorrhage was induced in six SHR after the end of a series of stimulations by bleeding through a catheter, introduced into the carotid artery. The BP level was measured periodically. Hemorrhage continued until BP fell to 50 mm Hg. The catheter was then reconnected to the electromanometer, and for the next 30 min approximately BP gradually rose, and when it stabilized at a level a little above 100 mm Hg, the same routine of measurements was repeated. The significance of differences was estimated by the T test, with an adopted level of significance of 5%. Correlation and regression analysis were carried out on a "Hewlett-Packard 10" computer by a preassigned program.

EXPERIMENTAL RESULTS

To compare the thickness of the microvascular wall in SHR and NR the vessels of the muscle were divided into groups in accordance with the diameter of the lumen: 5-9.9 μ, 10-14 μ,

Laboratory of General Pathology of the Microcirculation, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, I. K. Shkhvatsabaya.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 5, pp. 529-532, May, 1987. Original article submitted April 29, 1986.

 0007-4888/87/0005- 0598$12.50

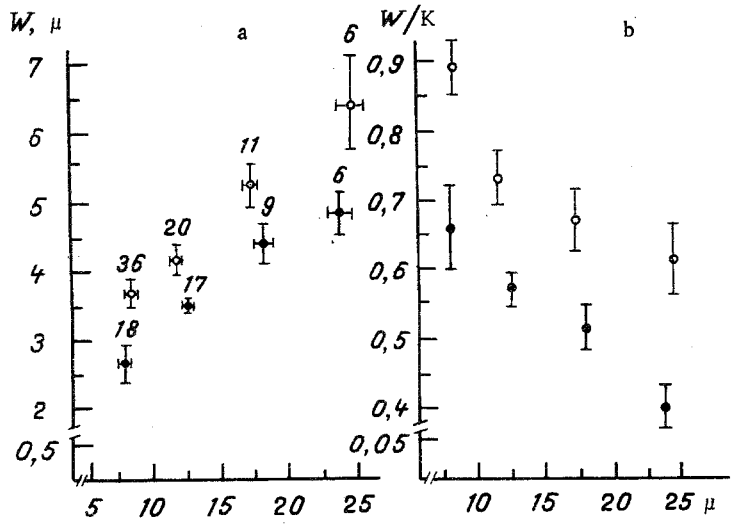

Fig. 1. Thickness (M ± m) of wall of different groups of vessels. Filled circles — NR (systolic BP 118 ± 4.26 mm Hg, n = 12); empty circles — SHR (systolic BP 171 ± 6.9 mm Hg, n = 10). a) Absolute thickness of wall; b) relative thickness of wall (w/r). Numbers indicate number of vessels.

15-19.9 μ, and 20-30 μ or more which, in accordance with the classification [4] for the rat cremaster muscle, corresponds approximately to arterioles of the 4th (4A), 3rd (3A), and 2nd (2A) groups; the two last groups of orders of branching. It will be clear from Fig. 1 that the absolute thickness of the microvascular wall of SHR was greater in all groups of vessels ($p < 0.01$), and with an incerase in diameter of the lumen there was a regular increase in thickness of the vascular wall in both NR and SHR. Correspondingly the ratio w/r (Fig. 1b) fell just as regularly, but its value also was higher in SHR.

Intravital microscopy of EHR thus revealed an increase of about 30% in the thickness of the vascular wall in SHR compared with this parameter in NR. However, it cannot be concluded from these results whether hypertropy of the smooth-muscle layer of the vessel wall took place in this case [5], whether the collagen component was increased [10], or whether functional vasoconstriction, together with structural changes, maintained the high peripheral resistance of the terminal vascular bed in SHR [8, 11].

Slowing of the blood flow and enlargement of the lumen were observed 3-5 sec after stimulation of the sympathetic chain in the microvessels of the muscle. Just as in the writers' previous investigation [3], the systemic BP did not differ significantly from its initial level. The experimental results given in Fig. 2 indicate that the reactivity of the microvessels in SHR was only about half that in NR.

The character of the relationship between v/w and ΔD% was found to be expressed by a straight line: $t_\gamma << 3$ both for NR and for SHR (γ is the index of linearity of association of η^2 (correlation ratio) and R^2 (coefficient of correlation). According to [1], $t_\gamma = \gamma/m_\gamma$ and if its value is below 3, correlation between the features is virtually linear. For NR R between w/r (but not w) and ΔD% was 0.69 ($n = 40$, $p < 0.01$), whereas for SHR, $R = 0.62$ ($n = 40$, $p < 0.01$). The almost identical value of R for NR and SHR will be noted. Regression lines plotted on the basis of the corresponding regression equations (Fig. 3) form angles close to 90° with the X axis (w/r), as is shown by the high coefficients of regression in both equations. This is evidence of a marked increase in ΔD% during a change in the relative unit w/r. The reactivity of the microvessels of the skeletal muscle of SHR to the neurogenic vasoconstrictor stimulus was thus depressed not only in absolute terms, but also because of the smaller degree of its increase accompanying an increase in w/r.

After hemorrhage BP of the SHR was established at the 119 ± 3.4 mm Hg level, which is about the same as BP in NR. In this case the vessels were not subdivided into groups, but only the over-all value of w/r = 0.9 ± 0.24 (n = 18) was determined. The results of this series of experiments are given in Fig. 2. The degree of vasoconstriction in response to a stimulus of equal strength had a clear tendency to rise, but it did not differ significantly

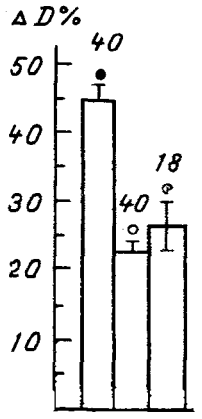

Fig. 2

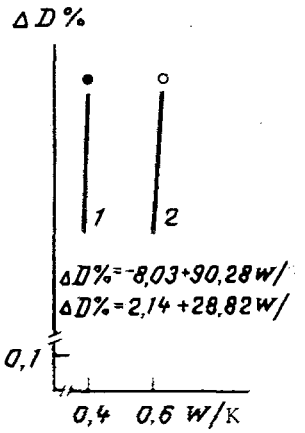

Fig. 3

Fig. 2. Effectiveness of sympathetic vasoconstrictor influence in NR, SHR, and SHR after hemorrhage (incomplete circle). Ordinate, changes in diameter of lumen of arterioles during stimulation of sympathetic chain (in % of initial value). Remainder of legend as to Fig. 1.

Fig. 3. Effectiveness of vasoconstrictor influences as a function of ratio w/r. Regression line and corresponding equations: $\Delta D\% = 2.14 + 28.82\ w/r$ (filled circle); $\Delta D\% = -8.03 + 90.28\ w/r$ (empty circle). Abscissa, ratio of thickness of wall to radius of vessel; ordinate, changes in diameter of lumen of arteroles during stimulation of sympathetic chain (in % of initial values). Remainder of legend as to Fig. 1.

from ΔD% in SHR before hemorrhage. The linear relationship between w/r and ΔD% was observed to be a little disturbed: $t_\gamma = 2.37$. Hemorrhage evidently potentiated vasoconstriction in the microvascular bed in response to stimulation of the sympathetic chain, and the intravascular pressure is evidently a decisive factor in this process.

In our view the lower reactivity of the skeletal muscular arterioles of SHR, discovered in these experiments, may be due not only to the higher intravascular pressure than in NR [4]. Hypertrophy of collagen tissues, rather than of muscle tissue, and the reduction in the myosin content in the vascular wall [3] may evidently be the reasons for the reduced reactivity of the microvessels, even though the wall was thickened.

It can also be postulated that the tangential tension (S_t) in the call of the skeletal muscular arterioles of SHR, just as in the microvessels of the mesentery [9], is 120-140% higher than in NR, and it lies outside the range of values of S_t at which the response to neurogenic vasoconstrictor stimuli is maximal [6]. Meanwhile, in the present experiments $w_{SHR} > w_{NR}$ by about 30-35% (a little more than 2A, see Fig. 1a) and, assuming that the intervascular pressure is higher in the arterioles of SHR than of NR by the same ratio as in the cremaster muscle [4], i.e., by 30-35%, it follows that S_t in SHR in the present experiments was about the same as in NR in vessels of the same diameter.

To explain the results we make use of theoretical considerations put forward to interpret earlier experimental data [2]. In experiments with perfusion of organs and tissues the response to the same stimulus (maximal stimulation of vasoconstrictor fibers, which was used in the present experiments also) was lower under constant flow than under constant pressure conditions. Hence it can be concluded that perfusion of the tissues of SHR takes place under conditions close to those of constant volume perfusion, and in turn, this is maintained by a high perfusion pressure at the entrance to the microcirculatory bed. A protective effect of raised intravascular pressure may thus be formed in the chronic type of hypertension, and vasoconstrictor stimuli will be less effective than in normal animals.

LITERATURE CITED

1. G. F. Lakin, Biometrics [in Russian], 3rd Edition, Moscow (1980).
2. L. V. Nikitin and V. M. Khayutin, Fiziol. Zh. SSSR, 48, 967 (1962).

3. M. I. Timkina and T. T. Yusupov, Byull. Eksp. Biol. Med., No. 8, 259 (1985).
4. H. G. Hohlen, R. W. Gore, and P. M. Hutchins, Microvasc. Res., 13, 125 (1977).
5. B. Folkow, M. Hallbäck, J. Lundgren, and L. Weiss, Acta Physiol. Scand., 79, 363 (1970).
6. R. W. Gore, Am. J. Physiol., 222, 82 (1972).
7. H. Henrich, R. Hertel, N. Kunzelmann, and A. Pfeilmeier, Pflügers Arch., 384, Suppl. R5 (1980).
8. H.-J. Herrmann, C. Norden, V. Moritz, and P. Mühlig, Int. J. Microcirc. Clin. Exp., 3, 483 (1984).
9. R. Hertel, H. Henrich, and R. Assmann, Experientia, 34, 865 (1978).
10. A. Ooshima, G. C. Fuller, G. J. Cardinale, et al., Proc. Natl. Acad. Sci. USA, 71, 3019 (1974).
11. J. W. Roy and H. N. Mayrovitz, Hypertension, 4, 264 (1982).
12. C. A. Sueta, P. M. Hutchins, and J. W. Dusseau, Hypertension, 5, 321 (1983).
13. R. J. Winquist and D. F. Bohr, Hypertension, 5, 292 (1983).

EFFECT OF VAGOTOMY, α-TOCOPHEROL, AND ARACHIDENE ON LIPID PEROXIDATION IN DIFFERENT PARTS OF THE GASTORDUODENAL ZONE IN RATS WITH EXPERIMENTAL PEPTIC ULCER

S. A. Morenkova, T. U. Tabutsadze, L. M. Fedorova, G. I. Myagkova, and R. P. Evstigneeva

UDC 616+342-002.44-089:616.833.191-089.85+615.356:577.161.3/-07:616.33/.342-008.930.15.39

KEY WORDS: lipid peroxidation, peptic ulcer, vagotomy, antioxidants, arachidene.

Activation of lipid peroxidation (LPO) is one of the leading factors reducing the resistance of the mucous membrane of the gastroduodenal zone [3, 6, 7]. LPO is initiated by stress stimulations [8] and is often the result of a deficiency of antioxidants in the tissues [5]. There is no doubt about the fact that various products of free-radical reactions are the cause of damage to the integrity of the cell membranes and, consequently, of the viability of the cell as a whole [4]. In this connection it can be postulated that disturbance of regulation of LPO may play an essential role in the pathogenesis of peptic ulcer.

The aim of this investigation was to study the role of LPO in experimental peptic ulcer and the effect of vagotomy, antioxidants, and arachidene on this process.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 140-160 g. Duodenal ulcer (DU) was induced by a single subcutaneous injection of cysteamine (Fluka, Switzerland) in a dose of 30 mg/100 g by the method in [14]. There were four groups of animals. Rats of group 1, 24 h after receiving the injection of cysteamine, underwent one of three versions of subdiaphragmatic vagotomy: 1) complete truncal vagotomy (CTV); 2) incomplete — division of one vagus trunk (ITV); and 3) proximal selective division of separate branches of the vagus nerve (PSV) by the method in [11]. The mucous membrane of the fundal and antral portions of the stomach and of the duodenum was removed 2 weeks after the operation and fixed in liquid nitrogen until required for use. Before receiving the injection of cysteamine the rats of group 2 were given daily subcutaneous injections of α-tocopherol (TP) in a dose of 5 mg/100 g for 5 days. For 5 days before the injection of cysteamine, rats of group 3 were given 0.1 ml of arachidene daily. Arachidene is a mixture of polyunsaturated fatty acids, which was synthesized in the Laboratory of the M. I. Lomonosov Moscow Institute of Fine Chemical Technology, directed by Corresponding Member of the Academy of Sciences of the USSR Professor R. P.

Laboratory of Biochemistry, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 5, pp. 532-534, May, 1987. Original article submitted September 30, 1986.

0007-4888/87/0005-0601 $12.50