Noninvasive Study of Vasodilatory Activity of Albino Rat Cerebral Arteries

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Vasodilating activity of rat cerebral vessels was studied. Methodological peculiarities and chronotropic limits of vasomotor testing were determined. Qualitative and quantitative parameters of dilatation of vessels were determined and similarity of rat dilatation responses and human vasodilatory activity was described.

Key Words: *rat; cerebral blood flow; vasodilatation; magnetic resonance tomography*

Vasomotor activity of vessels is now regarded as the main marker of functional competence of local mechanisms maintaining circulatory homeostasis [1,6]. The majority of methods for evaluation of the vasomotor function can be used only for studies of the peripheral compartments of the circulatory systems, which is explained by poor accessibility of cerebral areas for visualization [2]. On the other hand, the cerebral circulation determines the formation and prognosis for the majority of cardiovascular diseases [3,4]. In fact, no noninvasive methods for dynamic testing of the cerebral blood flow were described.

We determined methodological parameters of noninvasive testing of dilatation activity of rat intracerebral arteries.

MATERIALS AND METHODS

Experiments were carried out on 20 male Wistar rats (200-300 g). The animals were kept under standard vivarium conditions at natural illumination and free access to water and food. The groups consisted of 10 animals. Nitroglycerin (0.15 mg/kg) served as the positive control (as a drug allowed for use).

Before scanning the animals were narcotized with xylazinum (SPORA) in a concentration of 1 mg/ml and immobilized with relanium solution (2 mg/ml, intraperitoneally). Magnetic resonance tomography (MRT) was carried out in dynamics. Vessels were scanned on a PharmaScan US 70/16 tomographer for experimental studies (Bruker) at magnetic field strenght 7 T, frequency 300 MHz, and BGA 09P type coil. After tentative imaging in the axial, frontal, and sagittal planes, the main study was carried out in two projections (axial and frontal). T2-Weighed images were obtained in the spin echo (SE) mode using RARE_8 protocols adapted for the present study. The main image was obtained in the FL2D_ANGIO mode with the following parameters: 128×128 matrix, 1-mm thick section, 40 sections, 4×4 cm^2 reviewed area. MIP reconstruction was then carried out for vessel imaging.

Anatomic topographic structure of the cerebral blood vessels was evaluated by MRT. Serial sections in the frontal, sagittal, and horizontal planes with T2 tomograms and proton density weighed tomograms were made out using pulsed RARE_8, MSME, and GEFI sequences. Occipital 3D-construction of the vascular basin was made using Para Vision 3.0 licensed software (Bruker).

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TABLE 1. Vasodilatation of Cerebral Arteries

Parameter	Anterior cerebral	Middle cerebral	Posterior cerebral
Initial diameter of artery, mm	0.88 (0.37; 0.61-1.00)	0.89 (0.38; 0.62-1.02)	0.87 (0.35; 0.6-1.0)
	0.88 (0.38; 0.61-1.01)	0.87 (0.34; 0.61-1.00)	0.87 (0.35; 0.62-1.01)
Arterial diameter during nitroglycerin test, mm	1.22 (0.57; 0.89-1.61)	1.27 (0.61; 0.87-1.69)	1.21 (0.53; 0.96-1.71)
	1.23 (0.55; 0.91-1.58)	1.25 (0.58; 0.90-1.61)	1.22 (0.52; 0.95-1.69)
Nitroglycerin-induced dilatation, %	32.81±2.93	33.93±2.71	32.33±2.51
Initial signal intensity integral, units	3.92 (1.43; 3.42-4.51)	3.93 (1.31; 3.31-4.52)	3.92 (1.33; 3.43-4.24)
	3.81 (1.42; 3.21-4.42)	3.84 (1.43; 3.31-4.31)	3.92 (1.43; 3.51-4.43)
Signal intensity integral during nitroglycerin test, units	3.42 (1.21; 2.83-3.91)	3.41 (1.31; 2.51-3.64)	3.44 (1.12; 2.71-3.54)
	3.41 (1.14; 2.81-3.82)	3.43 (1.23; 2.65-3.71)	3.42 (1.26; 2.85-3.63)

Note. Mean values are presented (standard deviation; range of values). Numerator: left, denominator: right cerebral artery.

Vasodilating response was studied after sublingual dose of nitroglycerin solution. The diameters of cerebral arteries at the interface between the median and adventitial layers of the lateral and medial wall in the immediate vicinity of the selected anatomical marker were measured by two independent operators. The registration was carried out by the linear method, consisting in direct measurement of the artery diameter using two points established by the MRT cursor: at the adventitial-median interface on the lateral wall of the artery and at the median/adventitial interface on the medial wall (Fig. 1).

In all cases each operator estimated the nitroglycerin-dependent vasodilatation as the ratio of arterial diameter alteration during the test to its diameter at rest. These measurements were presented as percent ratio in comparison with the initial diameter, taken as 100%.

In order to verify the measurements carried out by the linear methods, computed estimation of the signal intensity at a preset square of the rat brain tomogram was carried out before and after nitroglycerin test and presented as histograms and statistical values (Fig. 2).

The reproducibility of the method by different operators was presented for all values as the arithmetic mean of the difference between the values obtained by different operators, standard deviation, range of values, and coefficient of variations. The relationship between the parameters was evaluated using linear correlation coefficient, the significance of differences was evaluated using Wilcoxon nonparametric test for independent samples and Wilcoxon—Mann—Whitney inversion U test.

RESULTS

High quality image of the cerebral basin was obtained, due to which it was possible to evaluate the arterial diameters and estimate the percentage of nitroglycerin-induced dilatation in all cases.

We measured the initial diameters of the anterior, middle, and posterior cerebral arteries of the left and right hemispheres by the linear method and estimated signal intensity for these regions (Table 1).

Sublingual treatment with nitroglycerin was accompanied by clinical effects of organic nitrates within 1-2 min and caused a significant dilation of cerebral arteries persisting for 30 min ($p<0.001$). The mean value of nitroglycerin-induced dilatation of cerebral vessels was 33.02±2.83% (Table 1). The signal intensity integral decreased by 13.74±0.92% ($p<0.001$) during nitroglycerin test.

The reproducibility of measurements was presented on a sample of 10 rats. A total of 240 mea-

TABLE 2. Results of Estimation of Reproducibility for Two Operators

Parameter	Number of measurements	Mean value, %	Standard deviation	Coefficient of variation, %
Nitroglycerin-induced dilatation	240	2.51	0.14	1.34
Initial diameter	120	1.23	0.11	1.21
Diameter during test	120	1.42	0.13	1.23

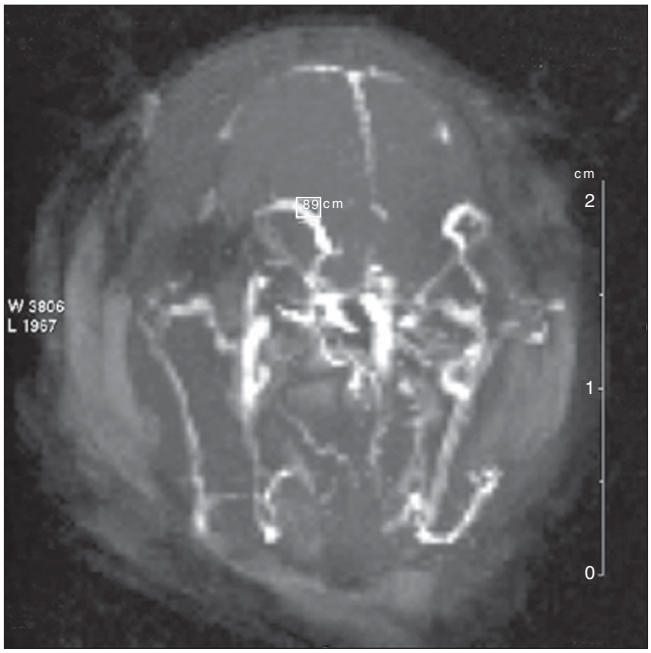

Fig. 1. Linear measurement of left anterior cerebral artery diameter.

surements were made (two examinations, 2 independent operators, measurements of arterial diameter in 2 tests, the mean diameter was estimated from 3 measurements). The mean difference in nitroglycerin-induced vasodilatation according to measurements made by different operators was 2.51± 0.01% (0-9), coefficient of variations being 1.34% (Table 2).

Analysis of correlations showed an inverse relationship between the degree of nitroglycerin-induced vasodilatation and initial diameter of the artery (r=-0.62) for the linear method and direct relationship of the former parameter and signal intensity integral (r=0.71) for computed estimation (p<0.01). No significant correlation between the degree of dilatation and cerebral area of the vascular basin was detected.

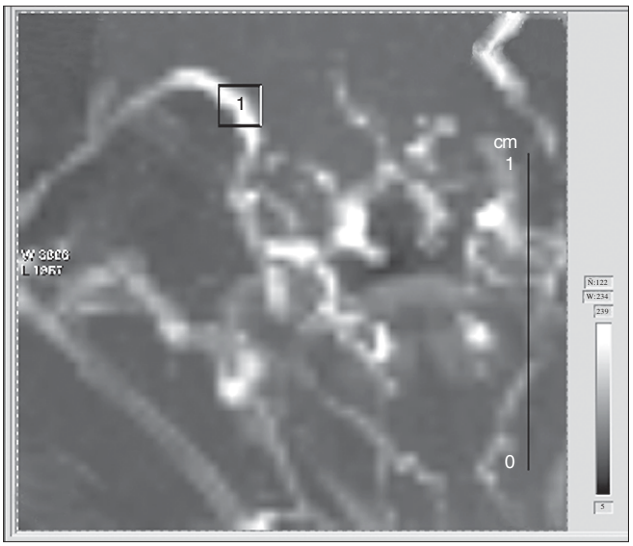

Fig. 2. Computed estimation of signal intensity integral in the anterior cerebral artery projection in a preset square.

Cerebral circulation largely determines the quality of life and the life span; its impairment is a priority cause of stable disability and lethal outcome [3].

The study of the anatomic topographic structure of the cerebral blood vessels in rats showed the absence of interhemispheric asymmetry of the cerebral blood flow in normal animals, which was confirmed previously [6].

The skull of an adult mammal is rigid and the brain is virtually incompressible, the total volume of brain tissue, cerebrospinal fluid, and blood in the intracranial vessels being almost constant. However, cranial blood flow can increase as a result of dilatation of the cerebral arteries. The minor increase in the volume of the brain is easily compensated by a minor stenosis of the veins, in which blood content is much greater than in the arteries. Sufficient vasodilatation of intracerebral arteries compensates for the drop of systemic blood pressure and maintains constant perfusion pressure in the major arteries of the brain [5]. Impairment of the system of intracerebral arteries vasodilatation is associated with cerebral ischemia, which was confirmed by experiments on rats with spontaneous hypertension; hypotensive drugs correct these shifts [3,6].

Magnetic imaging showed direct pharmacological effect of organic nitrates on cerebral vessels, associated with vasodilatation and increase in cerebral blood flow, which was confirmed by experimental findings in rats and in humans [3,4].

Evaluation of operators' errors showed that the method of noninvasive measurement of vasodilating function of the cerebral vessels is reproducible and its results are representative. The time course of nitroglycerin-induced vasodilatation, obtained by linear measurements of vascular diameter, precisely corresponded to the decrease in the computer-estimated signal intensity integral (r=-0.92; p<0.001), which indicates the possibility of separate use of each method.

Hence, noninvasive study of vasodilating function of cerebral vessels by MRT qualitatively and quantitatively shows the degree of relaxation of the cerebral arteries and is recommended for monitoring the endothelial dysfunction of cerebral vessels in cardiovascular diseases and for evaluating the efficiency of its drug correction.

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