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Transcranial Evaluation of Blood Flow Velocity in the Basilar Artery in Rats by High Frequency Ultrasonic Doppler Technique

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The possibility of recording the blood flow velocity in the basilar artery (*a. basilaris*) in rats through the occipital membrane by a two-channel ultrasonic Doppler system with single-element transducers was demonstrated. *A. basilaris* was located with a contact transducer working at a frequency of 33 MHz in two modes: pulsed and permanent. The mean blood flow velocity in narcotized male Wistar rats was 4.93±0.78 cm/sec. Functional loading tests (acute elevation of blood pressure and occlusion of the common carotid arteries) showed that this method allows recording dynamic characteristics of the blood flow reactions in *a. basilaris* with evaluation of its pulse wave pattern and analyzing their correlations with blood flow parameters in the carotid arteries using simultaneously the second (bandage) transducer.

Key Words: *ultrasonic Doppler methods; blood flow velocity; rat cerebral vessels; a. basilaris*

Evaluation of brain function and study of the compensatory adaptive mechanisms of the cerebral blood flow in functional tests provide the data on adequacy of cerebral hemodynamics to energy requirements of the brain tissue and are essential for defining the indications for adequate treatment of cerebrovascular abnormalities and for predicting the disease course and the effects of reconstructive interventions [1]. Animal experiments provide more data on the mechanisms of injury and approaches to therapeutic regulation of the cerebrovascular tone, on the pharmacological effects and mechanisms of action of drugs and their clinical potentialities [7]. The First (Foundation) Workshop of the Microcirculation and Regional Hemodynamics Society of the Russian Federation, affiliated to Association of Cardiovascular Surgeons of Russia (February 5-6, 2009, Moscow) adopted a decision to "recommend the method of high frequency dopplerography to companies manufacturing drugs, bioactive additives, therapeutic and functional nutrition for monitoring the efficiency and quality of innovations and products" [5]. Hence, the search for new possible applications of this method for evaluation of the cerebral hemodynamics in laboratory animals seems to be an important problem. It can be used for evaluation of blood flow velocity in the cerebral basilar arteries without violation of skull intactness and the volume/pressure ratio, because the reactions of the intracranial vessels and the cerebral circulation are closely linked with the liquor circulation.

In clinical practice, the ultrasonic signal of the cerebral arteries is recorded through the skull bones

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(transcranial ultrasonic dopplerography) with a transducer working at a frequency of 2 MHz and radiation energy of 100-200 W/cm^2 through four "ultrasonic windows": transtemporal, transorbital, transoccipital, and submandibular approaches [2]. These transducers are characterized by high penetration capacity, but relatively low sensitivity. The development of miniature single-element transducers working at frequencies above 38 MHz with lower radiation energy and fitted with an acoustic lens made it possible to focus the ultrasonic flow into a narrow (100-250 μ) beam capable of passing through narrow clefts and record the blood flow in individual microvessels at 3-5 mm/sec velocity [3,4]. The use of these transducers for noninvasive transcranial evaluation of the cerebral blood flow is fraught with difficulties, because of significant attenuation (poor penetrating capacity) of high frequency ultrasonic wave in tissues. Registration of blood flow velocity in rat cerebral basilar arteries by ultrasonic dopplerography with simultaneous color angioscanning was described only for the "open window in the skull" [8]. We used high-frequency ultrasonic Doppler system working at a frequency of 33 MHz for recording blood flow velocity in rat *a. basilaris* without opening the skull. We used an invasive methodology used for central injection through the occipital membrane into the cavity of the cerebral ventricle IV [6]. The aim of our experiment was to evaluate the efficiency of this approach in studies of the hemodynamics in the cerebral basilar arteries.

MATERIALS AND METHODS

Experiments were carried out on 20 male Wistar rats (320-380 g) under urethane narcosis (1.2 g/kg intraperitoneally) in accordance with Order No. 267 of the Ministry of Health of the Russian Federation of June 19, 2003 and with Regulations on Handling, Keeping, Analgesia, and Sacrifice of Experimental Animals, approved by the Ministry of Health of the USSR (1977) and Ministry of Health of Russian Soviet Federative Socialist Republic (1977). The animals were fixed in a stereotaxic device with the rostral part of their head positioned below the axis of the external acoustic meatuses. The skin with subcutaneous fat and the surface fascium on the occipital surface of the head were cut along the median line (15 mm). The suboccipital muscles were drawn apart with a blunt instrument in order to open the access to the occipital membrane.

The linear blood flow velocity (cm/sec) was recorded with a high-frequency ultrasonic Doppler two-channel system with single-element transducers created at the Bioengineering Laboratory of Institute of Pathology and Pathophysiology [3,4]. *A. basilaris* was located in the projection of the large occipital opening by applying the ultrasonic contact transducer (1.2 mm in diameter) suboccipitally with a micromanipulator directly to the posterior atlanto-occipital membrane at an angle of 40-60^o and orienting the ultrasonic beam to the sella turcica. A position in which the ultrasonic signal could be recorded with maximum intensity was found by moving the transducer in the central saggittal plane.

The transducer worked at a frequency of 33 MHz in two modes: permanent and pulsed. The depth of the basilar artery location was determined in the coherent pulsed radiation mode. In our studies this depth was 4.5-6.0 mm. The linear blood flow velocity was measured in the permanent ultrasonic radiation mode providing maximum level of Doppler signal. In parallel with pulsed blood flow, its mean velocity was measured using an analog device of the system and systemic blood pressure (BP) was recorded during the experiment with an electromanometer transducer inserted into the left femoral artery.

We used two standard approaches to find out whether this method is fit for studies of the mechanisms regulating the blood supply to brainstem structures under changing conditions of functioning and for evaluating the reactions to drugs. First, systemic BP was sharply elevated by intravenous injection of epinephrine hydrochloride (0.01 mg/kg) and second, blood supply to the brain was limited by clumping the common carotid arteries. The efficiency of occlusion was evaluated by changes in the blood flow velocity in the ipsilateral internal carotid artery recorded above the carotid sinus by a bandage ultrasonic transducer (inner diameter 0.5 mm, frequency 26.8 MHz) through the second channel of the ultrasonic system.

RESULTS

The linear blood flow velocity in *a. basilaris* recorded through the transoccipital access in narcotized rats was 4.93±0.78 cm/sec, which was slightly below the previously reported results obtained through the "triplex window" (5.9±1.2 cm/sec), but did not statistically differ from this value [8]. This minor difference was presumably due to different experimental conditions: different age and sex of animals, anesthesia, and consequences of craniotomy performed before registration. After injecting the maximum single dose of epinephrine, we observed the expected elevation of blood flow velocity in *a. basilaris* (Fig. 1). The blood flow passively followed the changes in systemic BP, indicating failure of the cerebral blood flow autoregulation reaction at the upper threshold. Increasing scanning velocity showed different pattern of the blood flow pulse wave in *a. basilaris* after norepinephrine effect was over, this indicating changed cardiac out-

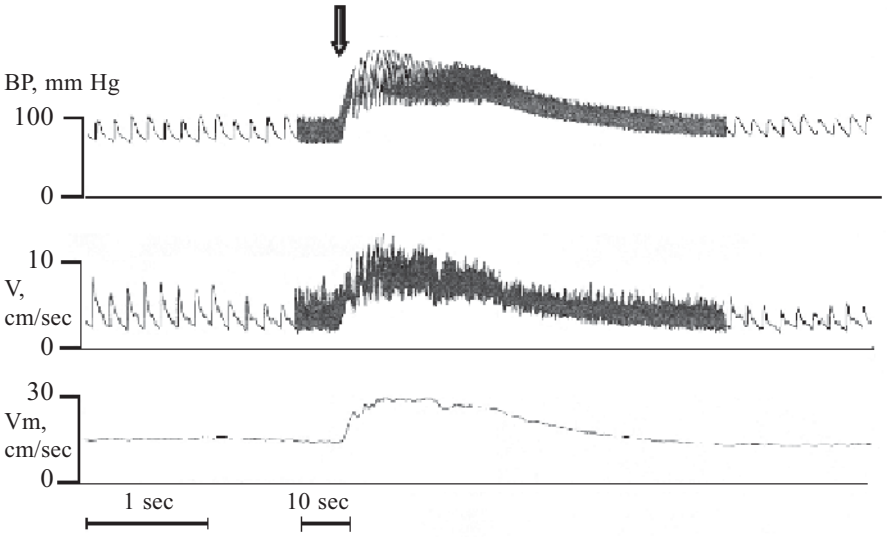

Fig. 1. Changes in systemic BP and blood flow velocity (V) in *a. basilaris* after intravenous epinephrine. Vm: mean blood flow velocity. Arrow shows epinephrine injection.

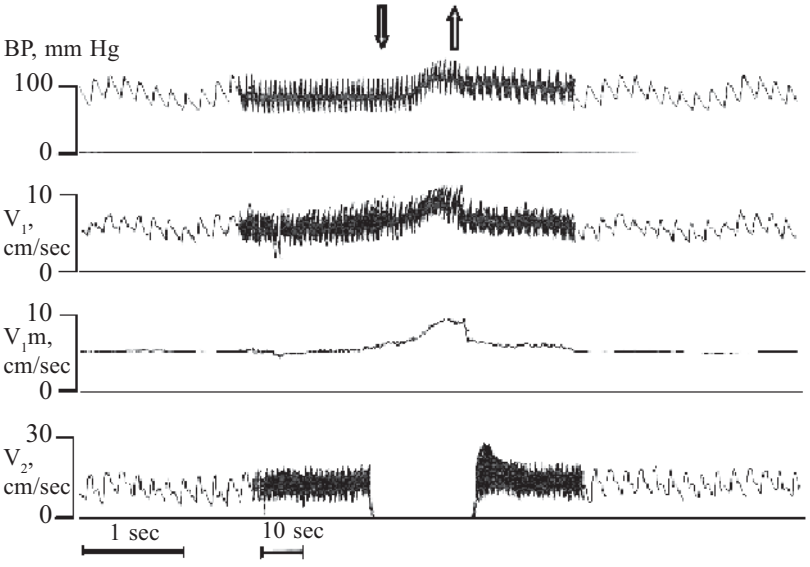

Fig. 2. Effects of common carotid artery occlusion on the parameters of blood flow velocity in *a. basilaris*. V_1: mean blood flow velocity in *a. basilaris*; V_1m: mean blood flow velocity in the *a. basilaris*; V_2: blood flow velocity in the ipsilateral internal carotid artery. Arrows show clipping of and removal of the clips from the common carotid artery.

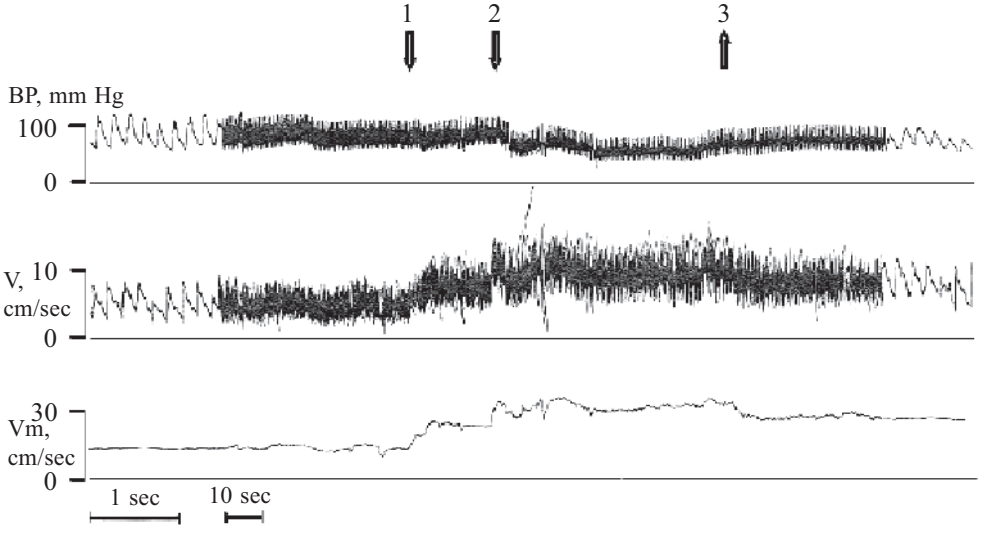

Fig. 3. Changes in systemic BP and blood flow velocity (V) in *a. basilaris* after step-by-step occlusion of the common carotid arteries. Vm: mean blood flow velocity. Arrows: *1*) clipping of one common carotid artery; *2*) clipping of the second common carotid artery; *3*) removal of the clips from the second common carotid artery.

put/vascular resistance proportion in the basin of this artery. In other experiments, occlusion of one (Fig. 2) and two (Fig. 3) carotid arteries was immediately followed by a compensatory increase in blood flow in *a. basilaris*. Differences in the pattern and intensity of the blood flow velocity changes were detected. In some animals, acceleration of the blood flow after occlusion was synphasic with BP shift, in others it occurred during the antiphase or against the background of unchanged BP. Depending on the number of clamped carotid arteries and duration of their occlusion, reperfusion after removal of the clips was paralleled by blood flow normalization or hyper- or hypoperfusion.

Hence, acute experiments on rats with sharp elevation of systemic BP and short-term limitation of the blood flow in the carotid arteries showed that our methodological approach can be used for recording the dynamic characteristics of blood flow reactions in *a. basilaris* to various factors, such as quantitative (cm/sec) and qualitative changes in the blood flow pulse wave pattern. Together with evaluation of the systemic hemodynamic parameters in the ascending aortic arch or in the carotid and cerebral arteries, this method can be highly informative. Its obvious advantage is the possibility of its repeated use for evaluation of the blood flow in the *a. basilaris* in one and the same animal in chronic experiments.

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