Role of Cholinergic Structures in Individual Resistance of Rat Circulatory System to Posthemorrhagic Hypoxia

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Experiments employing ultrasound technique showed that nonselective blockade of central muscarinic cholinoceptors with amizyl significantly increases the number and lifespan of rats highly resistant to acute massive blood loss. This pretreatment increased individual resistance of the circulatory system to posthemorrhagic hypoxia (blood pressure and portal blood flow rate). Preliminary blockade of central nicotinic cholinoceptors and peripheral muscarinic cholinoceptors with cyclodol and methacin, respectively, had no effect on the percentage of rats highly and low resistant to acute blood loss. Preliminary blockade of peripheral muscarinic cholinoceptors with methacin prevented the decrease in the cardiac output in low resistant animals during the posthemorrhagic period.

Key Words: *individual resistance to hypoxia; blood pressure; portal blood flow; cardiac output; cholinoceptor antagonists*

The individual systemic and cellular reactions to hypoxia of different genesis were extensively studied [1,3, 6,9]. It remains unclear which mechanisms determine individual resistance to oxygen deficiency at various integration levels. Our previous studies showed that preliminary blockade of rat $GABA_A$ receptors with picrotoxin significantly increases cardiovascular resistance to severe posthemorrhagic hypoxia [2]. GABAergic influences on the circulatory system are mediated by cholinergic structures in the bulbar cardiovascular center [11]. This work was designed to study whether preliminary blockade of central and peripheral cholinoceptors can modulate the resistance of rat circulatory system to severe posthemorrhagic hypoxia.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-280 g. The animals were intraperitoneally narcotized with urethane in a dose of 1.25 g/kg. They were divided into 4 groups. Group 1 included intact rats with acute massive blood loss (n=35). The rats of groups 2 (n=22), 3 (n=20), and 4 (n=24) intravenously received nonselective antagonist of central muscarinic cholinoceptors amizyl (1 mg/kg), antagonist of peripheral muscarinic cholinoceptors methacin (0.1 mg/kg), and antagonist of central nicotinic cholinoceptors cyclodol (1 mg/kg), respectively, 5 min before blood loss. Blood pressure in the femoral artery was measured with an electromanometer. Blood flow velocity and volume flow rate in the portal vein were estimated with a bandage ultrasound sensor after laparotomy [7]. The blood flow rate in the ascending aortic arch was recorded with an ultrasound catheter (diameter 0.6 mm) inserted through the carotid artery (without thoracotomy). Miniature piezocrystals operating at a frequency of 26.8 MHz served as a detecting element of the sensor [8]. Changes in the stroke and minute volume were recorded on an electronic device. In a special series respiratory movements of the thorax were registered with a tensiometric sensor.

Acute blood loss was produced by single blood effusion from the femoral vein (2.5% body weight) over 10 min. The period of observation depended on the lifespan of animals.

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The results were analyzed by Fischer and Student tests. The lifespan of animals after arrest of bleeding and posthemorrhagic changes in blood pressure and portal blood flow served as the criteria for individual resistance to acute blood loss.

RESULTS

Control rats exposed to blood loss were divided into groups of animals highly (HR, 62.9%) and low resistant to posthemorrhagic hypoxia (LR, 37.1%). It depended on the lifespan after arrest of bleeding and posthemorrhagic changes in blood pressure and portal blood flow rate. The lifespan of HR animals was 197.0± 21.3 min. Blood pressure and portal blood flow rate in HR rats decreased during blood loss, but temporarily returned to 70-80% of the basal level after arrest of bleeding (Fig. 1). In animals resistant to acute blood loss the posthemorrhagic period ran a typical course and included the stage of relative hemodynamic stabilization and irreversible secondary suppression (terminal phase). In LR rats the posthemorrhagic period was characterized by primary decompensation of blood pressure and portal blood flow and started from the terminal phase (Fig. 1). The lifespan of LR animals did not exceed 1.5 h.

As differentiated from variations in blood pressure and portal blood flow after blood loss, posthemorrhagic changes in cardiac output practically did not differ in HR and LR rats. In 100% HR rats and 61.6% LR animals, the aortic blood flow rate and stroke and minute volumes increased after arrest of bleeding and remained unchanged in the posthemorrhagic period. In 38.4% LR rats the irreversible primary decrease in blood pressure and portal blood flow rate was accompanied by reduction of cardiac output. It manifested in

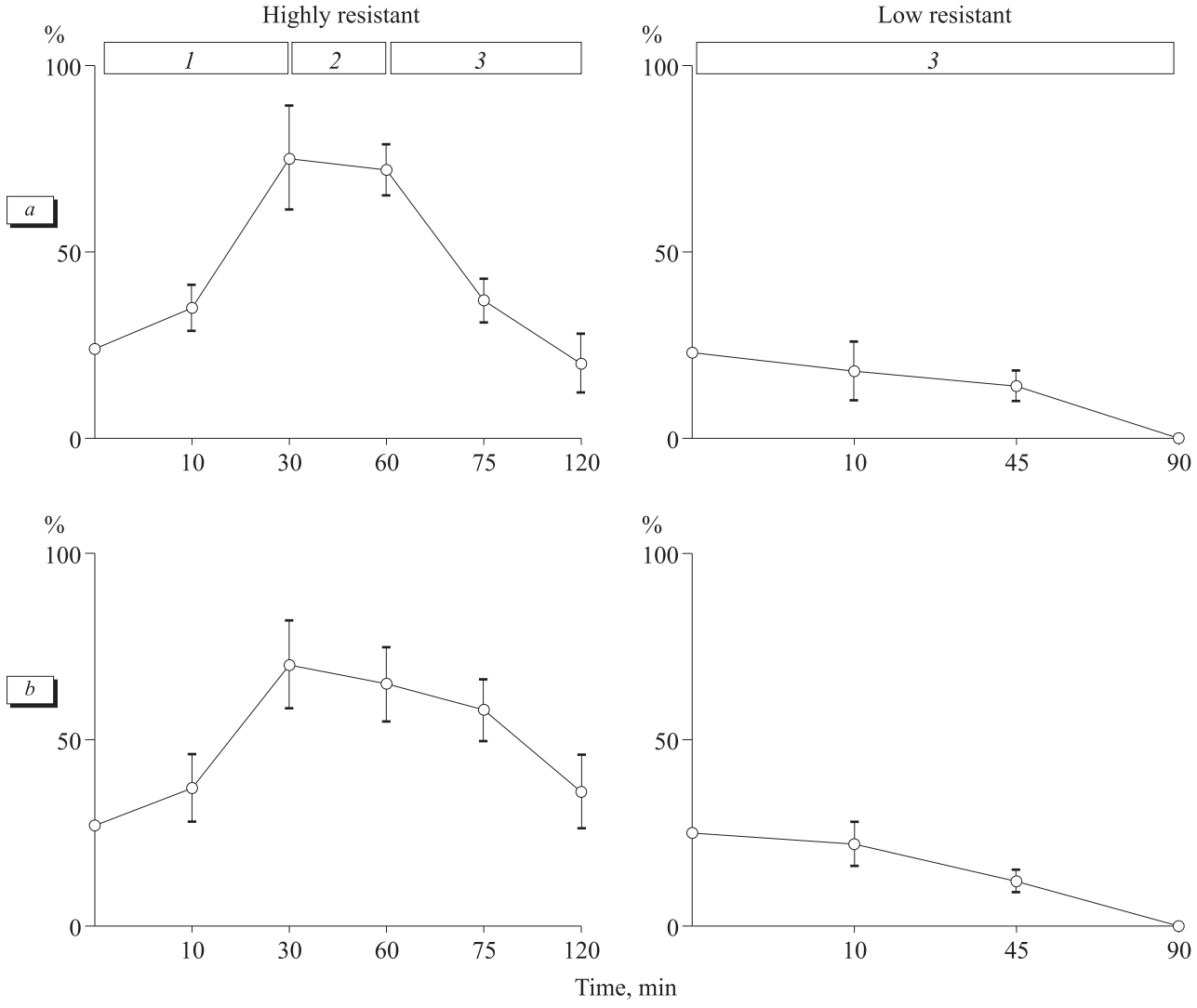

Fig. 1. Posthemorrhagic changes in systemic blood pressure (*a*) and volume rate of portal blood flow (*b*) in rats exhibiting high or low resistance to acute blood loss and exposed to acute massive blood loss. Recovery (*1*), compensatory (*2*), and terminal phase (*3*). Abscissa: time after arrest of bleeding. Ordinate: changes in the test parameter relative to the basal level (before blood loss).

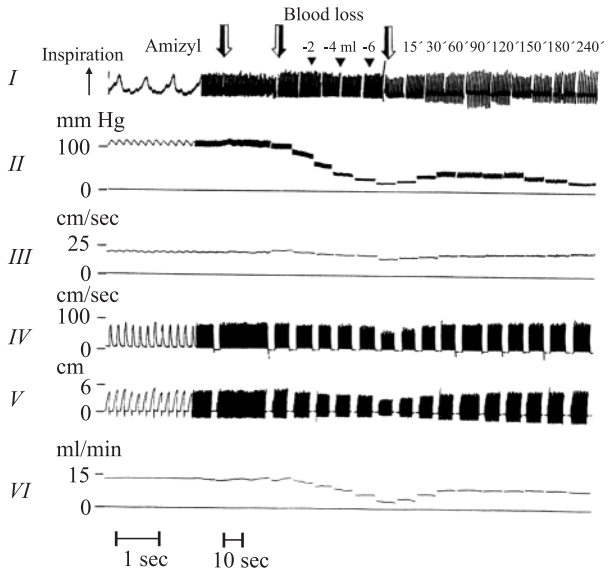

Fig. 2. Hemodynamics and respiration in the rat after preliminary blockade of central muscarinic cholinoceptors with amizyl (1 mg/kg intravenously, original curves). Here and in Fig. 3: respiration (*I*), systemic blood pressure (*II*), minute volume of blood (*III*), blood flow velocity in the ascending aortic arch (*IV*), stroke volume of blood (*V*), and volume flow rate in the hepatic portal vein (*VI*). First arrow: start of treatment with the preparation, time after the end of treatment (min). Second and third arrows: start and arrest of bleeding (ml). Follow-up period: time after arrest of bleeding (min).

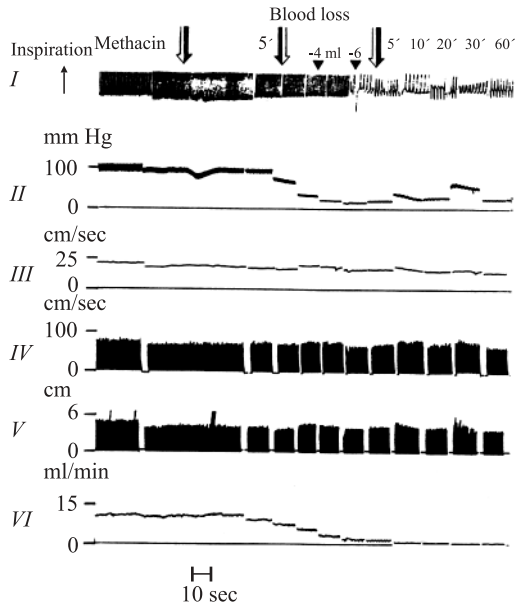

Fig. 3. Systemic circulation, portal blood flow, and respiration in the rat after preliminary blockade of peripheral muscarinic cholinoceptors with methacin (0.1 mg/kg intravenously, original curves).

a progressive decrease in aortic blood flow rate and stroke and minute volumes. These changes resulted in a fulminant course of the posthemorrhagic period and death of animals during blood loss or over the first 30 min after arrest of bleeding. Pretreatment with a nonselective antagonist of central muscarinic cholinoceptors amizyl (group 2) increased the number of HR rats with typical phasic course of the posthemorrhagic period to 90.9%. The lifespan of animals exposed to acute blood loss after pretreatment with amizyl increased to 299.5±35.2 min. These animals were characterized by lengthening of the phase for compensation of blood pressure and portal blood flow rate (Fig. 2). Primary decompensation of blood pressure and portal blood flow was observed in only 2 of 22 rats from group 2 during the posthemorrhagic period. Cardiac output in LR animals remained below the normal until death.

Preliminary blockade of peripheral muscarinic (methacin, group 3) and central nicotinic cholinoceptors (cyclodol, group 4) had no effect on the percentage of HR with posthemorrhagic phasic changes in blood pressure and portal blood flow. The ratio of LR rats with an irreversible primary decrease in these parameters remained unchanged. Blockade of peripheral muscarinic cholinoceptors in LR animals prevented the syndrome of reduced cardiac output after bleeding arrest (Fig. 3).

It can be hypothesized that central muscarinic cholinergic structures determine individual cardiovascular resistance to acute blood loss (blood pressure and portal blood flow). This assumption is consistent with published data that posthemorrhagic nonrecovery of blood pressure and portal blood flow and irreversible generalized posthemorrhagic constriction of microvessels in LR rats are associated with hyperactivation of central muscarinic cholinoceptors. It should be emphasized that nonselective cholinoceptor antagonist amizyl blocks central muscarinic cholinergic structures. Therefore, our results do not allow us to estimate which subtype of muscarinic cholinoceptors in the brain determines low resistance of the circulatory system to oxygen deficiency [12]. This problem can be solved in special experiments with selective antagonists of central muscarinic cholinoceptors. Our findings indicate that the syndrome of reduced cardiac output observed in 38.4% LR rats during the posthemorrhagic period is not mediated by central structures, but results from abnormal activation of peripheral muscarinic cholinoceptors in the heart. Pretreatment with peripheral muscarinic cholinoceptor antagonist methacin not crossing the blood-brain barrier prevented the posthemorrhagic syndrome of reduced cardiac output in LR animals.

We hypothesize that in LR animals strong afferentation from interoceptors of the heart and vessels [5] always accompanying acute massive blood loss led to the formation of a pathological system in muscarinic cholinergic structures of the brain regulating cardiac and vascular function. The concept of diseases postulates that this system formed under pathological con-

ditions sends abnormal pulses to target organs, which leads to the development of visceral disorders [4]. In LR rats these changes manifested in persistent irreversible generalized posthemorrhagic vasoconstriction, decrease in blood supply to organs, primary ischemia of organs, and posthemorrhagic nonrecovery of blood pressure [3]. The syndrome of reduced cardiac output develops in some LR rats. In most LR rats and all HR animals the heart is depleted of central nervous influences after arrest of bleeding (similarly to blockade of excitatory and inhibitory nervous impulses in the heart during severe hypoxia of different genesis) [10]. However, a posthemorrhagic failure of autoregulatory mechanisms in the heart is observed in 40% LR rats. It impairs blockade of central nervous influences on the heart and contributes to the syndrome of reduced cardiac output during severe hypoxia.

Our findings indicate that low individual resistance of the cardiovascular system to posthemorrhagic hypoxia can be attributed to dysregulation diseases.

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