Selective Blockade of Central M1 Muscarinic Cholinergic Receptors with Pirenzepine Impairs Cardiovascular and Respiratory Function in Rats with Acute Hemorrhage

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Ultrasound studies showed that selective antagonist of central M1 muscarinic cholinergic receptors pirenzepine (50 mg/kg intravenously) causes transitory hypotension and respiratory depression in anesthetized intact rats. The M1 receptor antagonist had no effect on cardiac output and portal blood flow. Pretreatment with pirenzepine increased the sensitivity of rats with acute massive hemorrhage to circulatory hypoxia. After blockade of central M1 muscarinic cholinergic receptors, the posthemorrhagic period was characterized by primary decompensation of blood pressure, portal blood flow, and respiration and development of low cardiac output syndrome. The animals died over the first minutes after bleeding arrest. Our results indicate that central M1 muscarinic cholinergic receptors act as shock-limiting cholinergic structures under conditions of posthemorrhagic changes in systemic and portal blood flow, as well as during respiratory dysfunction.

Key Words: *hemorrhage; blood circulation; respiration; pirenzepine; muscarinic receptors*

Shock and acute hemorrhage are accompanied by activation of the sympathetic and parasympathetic autonomic nervous systems. These changes include the increase in acetylcholine concentration, decrease in acetylcholine esterase activity in the blood and brain tissue, and activation of central muscarinic cholinergic receptors (M-CR). Published data show that activation of muscarinic cholinergic structures in the brain during shock is followed by inhibition of cardiovascular and respiratory function [1,3,10]. The role of various subtypes of central M-CR [9] in the pathogenesis of hemodynamic and respiratory dysfunction under shock conditions remains unknown.

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Here we studied the effect of blockade of cerebral M1-CR with highly selective antagonist pirenzepine on the circulatory system and respiratory function in rats with acute massive hemorrhage.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-280 g. The animals were intraperitoneally anesthetized with urethane in a dose of 1.25 mg/kg. The control group included intact rats with acute massive hemorrhage (n=19). The animals of treatment group 1 (n=16) intravenously received highly selective antagonist of central M1-CR (pirenzepine, 50 mg/kg) 15 min before bleeding. Pirenzepine in this dose crosses the blood-brain barrier [5]. Acute hemorrhage was produced by blood-

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letting from the femoral vein (2.5% body weight) over 10 min. The period of observation depended on animal lifespan after bleeding arrest. Blood pressure (BP) in the femoral artery was measured using an electromanometer. Blood flow velocity and volumetric flow rate in the hepatic portal vein were estimated using a bandage ultrasound sensor after laparotomy. The blood flow rate in the ascending aortic arch was recorded with an ultrasound catheter (diameter 0.6 mm) inserted through the right carotid artery (without thoracotomy). Miniature piezo-crystals operating at a frequency of 26.8 MHz served as the detecting elements in this sensor. Changes in stroke volume (SV) and cardiac output (CO) were recorded using an electronic device [6-8]. Respiratory movements of the thorax were recorded with a tensiometric sensor.

The results were analyzed by Fischer—Student test. The lifespan of animals after bleeding arrest and type of posthemorrhagic changes in BP and portal blood flow served as the criteria for individual resistance to acute hemorrhage [2].

RESULTS

BP and volumetric rate of portal blood flow in control animals rapidly decreased during acute hemorrhage. By the end of hemorrhage these parameters did not exceed 26.8±4.6 and 30.1±8.2% of the basal level, respectively. CO moderately decreased in most animals (not more than by 20-25%), but remained at the subnormal level in some animals.

By the end of hemorrhage, control animals were divided into 2 groups depending on the lifespan and type of posthemorrhagic changes. The animals with lifespan of 197.7±24.5 min, in whom bleeding arrest was followed by a phase of temporary BP and volumetric rate of portal blood recovery (to 72.4±12.5 mm Hg and 69.4±15.2% of the basal level, respectively), phase of relative stabilization, and phase of irreversible decrease in the test parameters, comprised the group of highly resistant animals (Fig. 1) and with compensated course of the posthemorrhagic period (63% animals). Other rats died over the first 1.5 h after bleeding arrest and were characterized by an irreversible decrease in BP and rate of portal blood flow during the posthemorrhagic period. They comprised the group of animals low resistant to circulatory hypoxia (Fig. 2) and exhibited decompensated course of the posthemorrhagic period (37% animals).

As distinct from posthemorrhagic changes in BP and portal blood flow, posthemorrhagic variations in CO were similar in highly resistant and

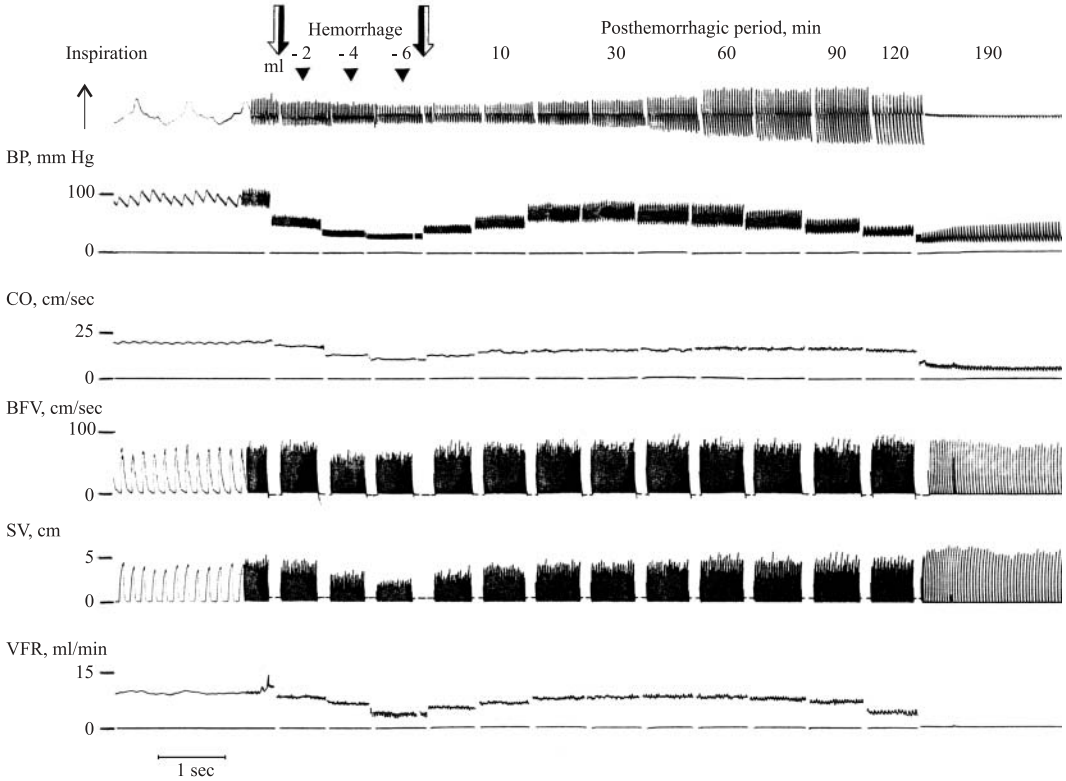

Fig. 1. Respiratory and circulatory response to acute hemorrhage in rats highly resistant and low resistant to circulatory hypoxia. Here and in Figs. 2 and 3: thin arrow, inspiration; double arrows, start and end of hemorrhage (ml). BFV, blood flow velocity in the ascending aortic arch; VFR, volumetric flow rate in the hepatic portal vein.

majority of low resistant animals. Aortic blood flow rate, SV, and CO in all highly resistant rats and 61% low resistant animals (Fig. 2) progressively increased after bleeding arrest and during the terminal phase of shock (until cessation of breathing). Hence, pump function of the heart in these animals was preserved to the end of their life. In 39% low resistant rats, primary irreversible posthemorrhagic decrease in BP and rate of portal blood flow was accompanied by the development of low CO syndrome. It manifested in a progressive decrease in aortic blood flow rate, SV, and CO. These changes resulted in fulminant course of the posthemorrhagic period and death of animals over the first 30 min after bleeding arrest. These data are consistent with the results of our previous experiments on intact animals with acute massive hemorrhage [2,3].

After hemorrhage the respiratory rate and amplitude of respiratory movements in rats decreased by 21.6±5.1 and 25.6±7.5%, respectively. These changes did not depend on the individual resistance of animals to posthemorrhagic hypoxia. The inhibition of respiratory function of the lungs increased in 68% highly resistant rats during the posthemorrhagic period. These changes resulted in irreversible cessation of breathing and subsequent cardiac arrest. In one third of highly resistant rats, the rate and amplitude of respiration returned to normal after bleeding arrest. In these rats the amplitude of respiration increased by 30-40%, while the respiratory rate decreased by 15-20% by the end of the compensation phase. These changed usually preceded the start of an irreversible decrease in BP, which is typical of the terminal phase. The amplitude of respiratory movements in these animals progressively increased in the follow-up period. The decrease in the respiratory rate resulted in cessation of breathing (Fig. 1).

The type of posthemorrhagic respiratory dysfunction was similar in all low resistant specimens. It manifested in a rapid decrease in the rate and amplitude of respiration to zero (Fig. 2).

Systemic administration of pirenzepine was followed by transitory changes in respiration and blood circulation in intact rats (Fig. 3). A 60-70% decrease in the amplitude and rate of respiration was observed 15 sec after intravenous injection of highly selective M-CR antagonist. BP decreased to 35-40 mm Hg. These parameters progressively returned to normal 1 min after pirenzepine admini-

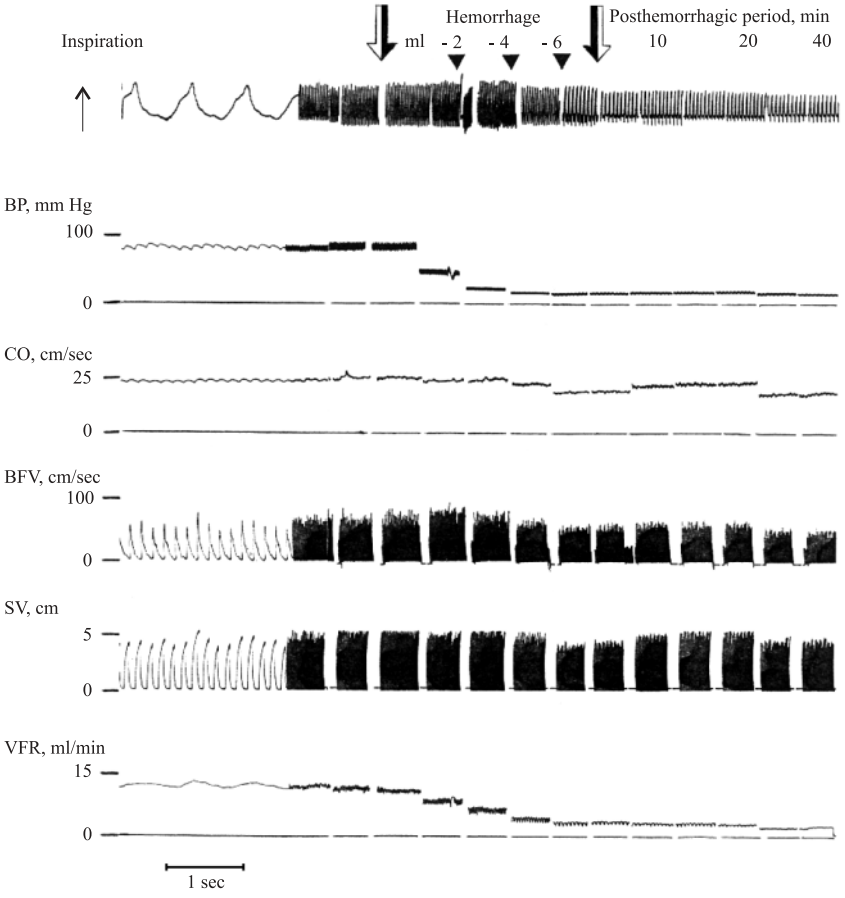

Fig. 2. Respiration, systemic hemodynamics, and portal blood flow in the rat low resistant to acute hemorrhage.

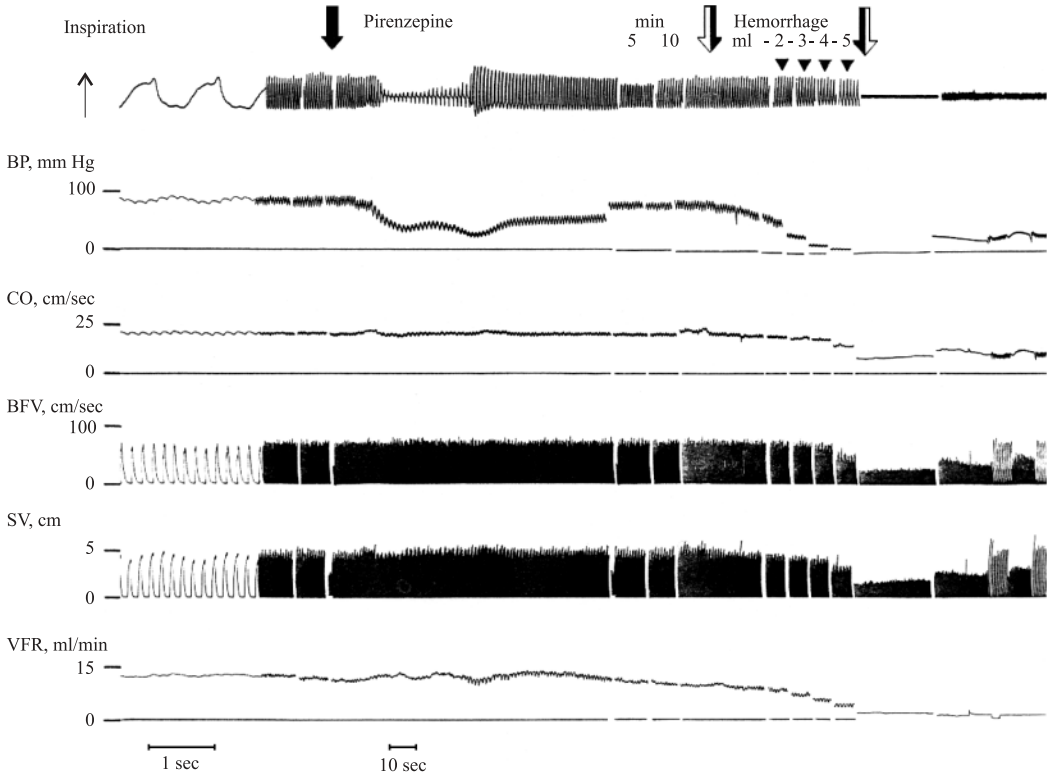

Fig. 3. Effect of pirenzepine (50 mg/kg intravenously) on respiration and blood flow in the rat under normal conditions and during acute hemorrhage. Dark arrow: start of drug administration; next segment, time after drug administration (min).

stration. An increase in the rate and amplitude of respiration preceded the elevation of BP by 5-10 sec. The amplitude of respiration was recovered in a stepwise manner (over 2-3 sec) and slightly exceeded the basal level. BP progressively returned to normal over 2-3 min after cholinolytic treatment. Pirenzepine had no effect on CO and volumetric rate of portal blood flow in intact animals.

The rats pretreated with pirenzepine had specific features of hemorrhage and posthemorrhagic period (Fig. 3). By the end of hemorrhage, respiratory dysfunction in animals with blockade of central M1-CR was more severe compared to control rats of the hemorrhage group. Variations in systemic and portal blood flow in these rats did not differ from the control. BP in rats receiving the selective antagonist decreased to 23.4±6.7 mm Hg after bleeding arrest. The aortic blood flow rate, SV, and CO decreased not more than by 20-25%. The volumetric rate of portal blood flow was 28.2±7.6% of the basal level. Irreversible cessation of breathing in treated animals developed rapidly after bleeding arrest. BP dropped to zero. The blood flow rate in the ascending aorta, CO, and SV decreased to 50-60%. The rate of portal blood flow did not exceed 20% of the basal level. The animals died over the first minutes after bleeding arrest.

Our results indicate that central M1-CR are involved in the regulation of systemic BP and respiration under normal conditions. Blockade of central cholinergic structures with highly selective antagonist pirenzepine causes transitory hypotension and respiratory depression in intact animals. However, blockade of these M-CR in the brain has no effect on CO and portal blood flow. Published data show that intravenous injection of pirenzepine prevents BP elevation and increase in the concentrations of norepinephrine and vasopressin in the plasma of intact dogs after intraventricular administration of acetylcholine. Therefore, the relationship exists between these cholinergic responses and activation of central M1-CR [11]. Prior selective blockade of central M1-CR increases the sensitivity to circulatory hypoxia during acute massive hemorrhage. Acute hemorrhage is accompanied by primary decompensation of BP, portal blood flow, and respiration and posthemorrhagic decrease in the pump function of the heart in animals with blockade of central M1-cholinergic structures. The lifespan of these rats after bleeding arrest does not exceed 5 min.

However, published data and results of our experiments show that the inhibition of respiratory and cardiovascular function during shock and acute

hemorrhage is associated with activation of central M-CR. Nonselective blockade with benactyzine has a negative effect on the course of shock [1,4,10]. These contradictory data indicate that various subtypes of central M-CR have different role in the pathogenesis of circulatory and respiratory dysfunction during acute hemorrhage. Central muscarinic structures are characterized by functional heterogeneity under conditions of posthemorrhagic hypotension and respiratory dysfunction. Central M1-CR act as shock-limiting, but not shock-potentiating cholinergic structures during acute hemorrhage. The correction of circulatory hypoxia should include activation, but not inhibition of these structures. Our results indirectly support published data that intraventricular administration of acetylcholine or its precursors during hemorrhagic and spinal shock is followed by the increase in systemic BP and development of reversible shock hypotension [12-14].

Cholinergic drugs with the optimum range of receptor activity hold much promise not only to study the pathogenesis of shock, but also to develop new pathogenetic schemes of treatment.

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