Role of Various Subtypes of Muscarinic Cholinergic Receptors in the Development of Posthemorrhagic Abnormalities in Systemic and Portal Circulation in Rats

N. Ya. Kovalenko, D. D. Matsievskii, and V. K. Reshetnyak

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 9, pp. 260-264, September, 2011
Original article submitted May 4, 2010

The experiments employing high-frequency ultrasonic technique and selective blockers of M1, M3, and M4 muscarinic cholinergic receptors pirenzepine, 4-DAMP, and tropicamide, respectively, revealed individual roles of these receptors in the development of severe posthemorrhagic hypotension in rats with low or high individual resistance to circulatory hypoxia. The study showed that M1 and M4 muscarinic receptors are involved in shock-limiting and shock-activating processes, respectively, while M3 receptors exert no effect on the course of posthemorrhagic abnormalities in systemic and hepatic portal circulation and on the posthemorrhagic lifespan. Poor resistance of the cardiovascular system to circulatory hypoxia during shock development is considered to be dysregulatory pathology.

Key Words: *M1, M3, M4 muscarinic cholinoreceptors; pirenzepine; 4-DAMP; tropicamide; acute hemorrhage*

During the last decades, various types of muscarinic cholinoreceptors (M-ChR) were found in brain structures responsible for central regulation of the cardiovascular system, in the heart, and in the walls of blood vessels. They differ in the matter of second messengers, tissue-related localization, and *in vitro* functional responses [8-12]. Despite extensive studies elucidating the above peculiarities of M-ChR at the molecular level, functional specificity of various subtypes of M-ChR and their role in the development of cardiovascular pathology were little examined. Specifically, the role of five presently known M-ChR subtypes in the pathogenesis of circulation system during shock and acute hemorrhage is still unclear.

We previously showed that blockade of M-ChR with nonselective cholinolytic amizylum (benactyzine) improved the posthemorrhagic condition of the cardiovascular system in rats with low and high resistance to circulatory hypoxia [1,2].

This study was designed to examine the role of M1-, M3-, and M4-ChR in the development of the posthemorrhagic disturbances in the cardiovascular system of the rats with low and high resistance against acute hemorrhage.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 250-280 g anesthetized with intraperitoneal urethane (1.25 g/kg). The animals were randomized into 4 groups. Group I (intact control animals) comprised untreated rats subjected to acute hemorrhage only (n=21). Ten minutes before acute hemorrhage, group II rats (n=15) were intravenously injected with 50 mg/kg pirenzepine (Sigma), a highly selective blocker of cerebral M1-ChR crossing the blood-brain

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** niiopp@mail.ru. D. D. Matsievskii

0007-4888/12/1523-0293

barrier [3]. Similarly, group III (n=17) and group IV (n=16) rats were injected with 0.2 mg/kg 4-DAMP (Tocris), a selective blocker of M3-ChR, and 0.0001 mg/kg tropicamide (Sigma), a selective blocker of M4-ChR, respectively. Systemic BP was recorded in the femoral artery with a micromanometer. Blood velocity and volume flow rate were determined in the portal vein after laparotomy by a cuff-type ultrasonic transducer with the internal diameter of 1.5 mm. The blood velocity in the ascending part of the aorta was measured in unopened thorax with a 0.6-mm ultrasonic catheter passed into carotid artery. This catheter was equipped with a miniature piezocrystals working at the frequencies of 26.8 and 33 MHz [5,6]. An electronic device was employed to measure the time course of cardiac output and stroke volume.

Acute hemorrhage was performed by a single 10-min bleeding from the femoral artery (2.5% body weight). The rats were observed to death. The data were processed statistically using Fisher-Student and Student's t test at p<0.05. The posthemorrhagic lifespan and changes in BP and hepatic portal circulation served as the measures of individual resistance to acute hemorrhage.

RESULTS

By posthemorrhagic lifespan and the dynamics of BP and portal circulation the animals after control acute hemorrhage were divided into two groups: with high and low resistance to posthemorrhagic hypoxia (61.9% and 38.1% animals, respectively). The lifespan of highly resistant rats was 185.0±23.2 min. In the posthemorrhagic period, we observed a transient increase BP and portal flow rate to (70-80% of initial level) after their drop in highly resistant rats (Fig. 1). Then these rats demonstrated the typical course of the posthemorrhagic period with a phase of relative stabilization of the studied hemodynamic indices followed by their secondary and irreversible decrease (terminatal phase). In contrast, the posthemorrhagic period in low resistant rats was characterized by primary decompensation of BP and hepatic portal circulation; it started immediately with the termination phase (Fig. 1). The lifespan of low resistant rats was no longer than 1.5 h. In contrast to the posthemorrhagic changes of BP and hepatic portal blood flow, the character of posthemorrhagic dynamics of cardiac output (CO) was similar

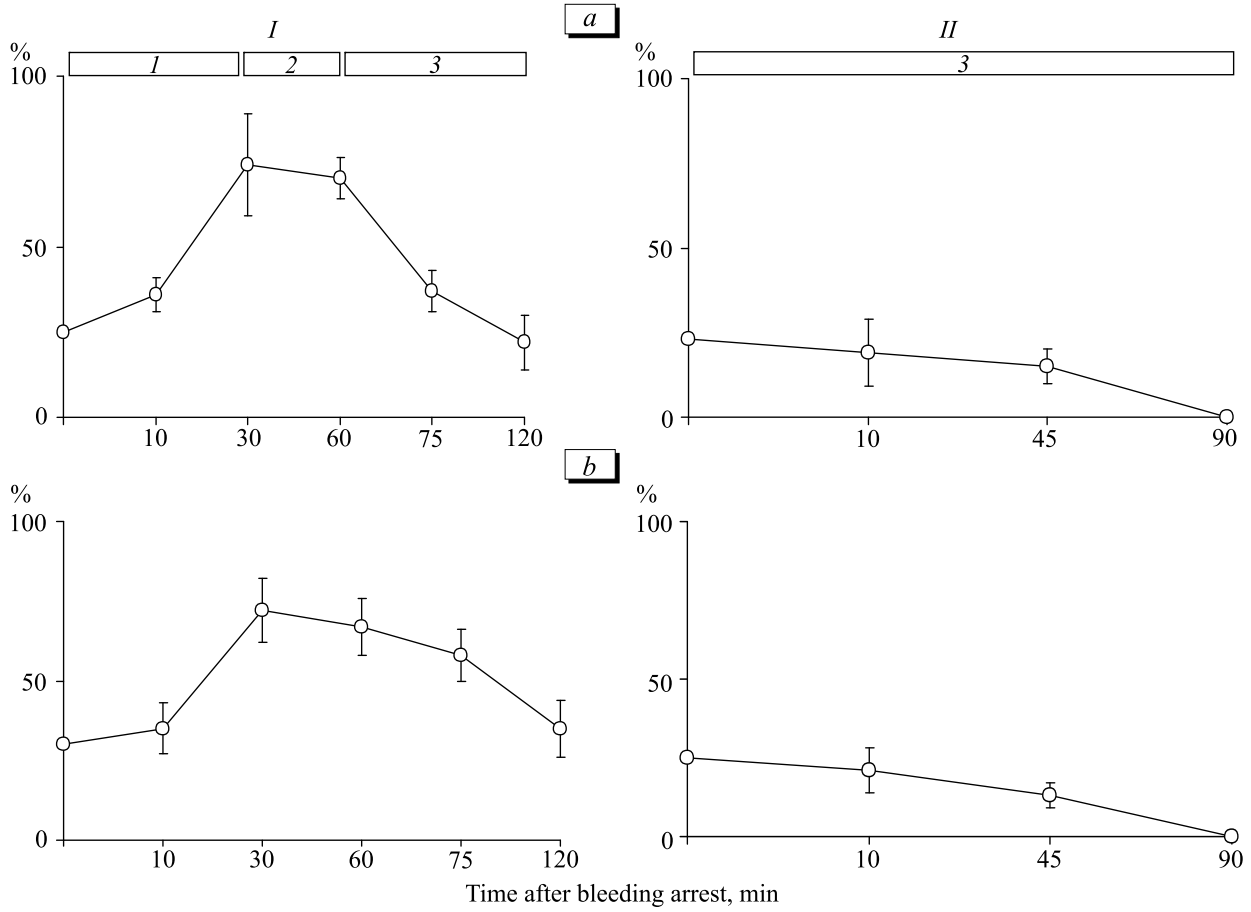

Fig. 1. Posthemorrhagic timing of systemic BP (*a*) and hepatic portal blood flow rate (*b*) in rats with high (*I*) and low (*II*) resistance against acute massive blood loss. *1*) restoration phase; *2*) compensatory phase; *3*) terminal phase. Ordinate: BP and flow rate data are given in percentage to prehemorrhagic levels.

in all highly resistant rats and in the majority of low resistant rats. In all highly resistant rats and 62.5% low resistant rats, the aortal blood velocity, stroke volume (SV), and CO of the heart increased after bleeding arrest, and this trend persisted during the terminal phase of the posthemorrhagic period. In 40.2% low resistant rats, low cardiac output syndrome developed against the background of immediate and irreversible drop of BP and decrease of portal flow rate. This syndrome was characterized by a progressive decrease in SV, CO, and aortal blood velocity resulting in malignant and rapid course of the posthemorrhagic period terminating with death during hemorrhagic period or within 30 min after bleeding arrest.

Intravenous injection of pirenzepine (a highly selective blocker of cerebral M1-ChR) to group II rats before hemorrhage induced the following transient changes in circulation. On second 15 postinjection, BP decreased to 35-40 mm Hg. Starting from the end of the first postinjection minute, BP gradually restored during 2-3 min. Thus, pirenzepine had no effect on CO and portal flow rate in intact rats (Fig. 2).

In group II rats treated with pirenzepine, the hemorrhagic and posthemorrhagic periods were characterized as follows. At the end of bleeding period, changes in systemic and regional (portal) circulation parameters did not differ from the similar changes in the control group. At the end of bleeding period in group II rats, BP decreased to 19.9±7.8 mm Hg; the aortal blood velocity, CO, and SV decreased to 24.8±7.9 mm by no more than 20-25%, while the portal flow rate decreased to 26.9±8.7% initial level. After bleeding arrest, rapid and irreversible drop of BP to virtually zero value was observed in all the rats of this group. At the same time, blood velocity in the ascending arch of the aorta, SV and CO decreased to 50-60% initial values. The portal flow rate did not exceed 20% of the initial level. The rats died during the first posthemorrhagic minutes.

In group III rats, injection of 4-DAMP (M3-ChR blocker) produced no significant effect on the hemodynamic parameters before hemorrhage and the dynamics of posthemorrhagic changes in comparison with the control group. In addition, this injection did not shift the ratio of the highly resistant rats (characterized by phasic posthemorrhagic changes in BP and portal flow rate) to the low resistant rats with initially irreversible decrease of these parameters.

In group IV rats, injection of tropicamide (M4-ChR blocker) produced a transient BP decrease by 20-25%. This blocker did not significantly affect CO and portal blood flow, but increased the percent of highly resistant rats to 87.5%. Moreover, tropicamide increased the posthemorrhagic lifespan to 275±34.6 min and significantly prolonged the compensatory phase (by BP and hepatic portal flow rate, Fig. 3). Only 2 of 16 rats in this group, the posthemorrhagic period was characterized by primarily irreversible decompensation of BP and portal circulation. In these

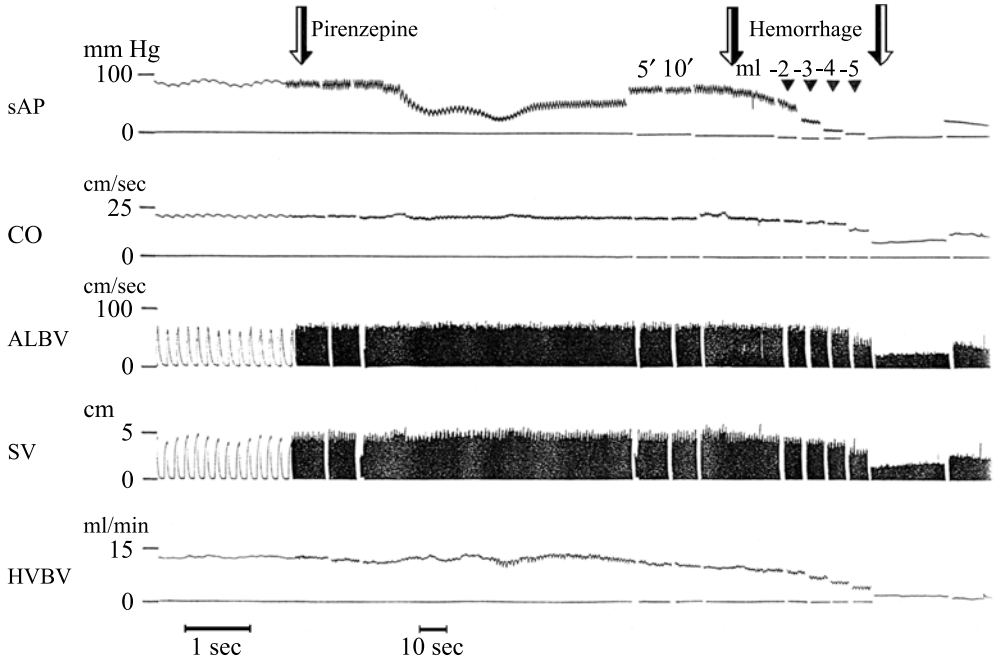

Fig. 2. Effect of pirenzepine (50 mg/kg *i.v.*) on systemic and regional (hepatic portal) circulation in norm and during acute hemorrhage in rats. The first arrow (the most left) marks the beginning of drug administration; 5' and 10' mark time (min) after termination of drug administration; the second and third arrows indicate the onset and the end of acute hemorrhage (the blood loss is shown in ml). Here and in Fig. 3: sAP, systemic arterial pressure; CO, cardiac output; ALBV, blood velocity in the ascending arch of the aorta; SV, stroke volume; HVBV, blood flow rate in hepatic portal vein.

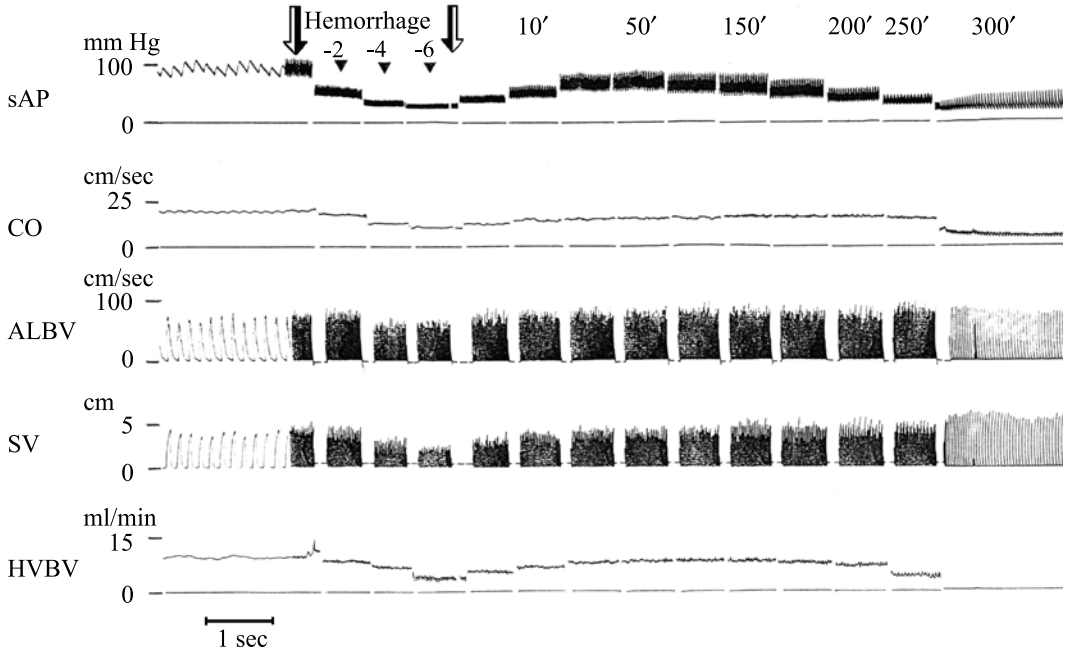

Fig. 3. Effect of preventive blockage of M4-ChR with tropicamide (0.0001 mg/kg *i.v.*) on systemic and regional (hepatic portal) circulation during and after acute hemorrhage in rats. The arrows mark the onset and the end of acute hemorrhage (blood loss is shown in ml). The posthemorrhagic time is shown in minutes.

low resistant rats, SV and CO remained below the norm until the death.

According to our previous data, inhibition of central M-ChR with a non-selective blocker amizylum alleviated the course of posthemorrhagic period assessed by systemic BP, hepatic circulation, and lifespan of all examined animals irrespective of their individual sensitivity to acute hemorrhage [1,2]. The highly selective muscarinic blockers used in the present study showed that various M-ChR subtypes are heterogenic structures involved in implementation of the opposite functions during the development of severe posthemorrhagic hypotension in the rats with high or low resistance against circulatory hypoxia. Indeed, preliminary selective blockade of cerebral M1-ChR with pirenzepine aggravated vulnerability of the organism to circulatory hypoxia. In all rats with blocked M1-ChR, acute hemorrhage resulted in primary decompensation of systemic BP, hepatic circulation, and irreversible impairment of the pumping function of the heart. In contrast, inhibition of M4-ChR improved the posthemorrhagic state of the cardiovascular system in rats with high and low resistance to acute hemorrhage. Thus, M1-ChR located in the brain [8] are probably the shock-limiting cholinoreactive structures. By contrast, M4-ChR located in the medulla oblongata and in the vascular wall [7,9,10] exert an antagonistic shock-activating effect. At the same time, M3-ChR which are the peripheral cholinoreactive structures, are present in endothelial cells of virtually all organs, are responsible for the non-neural endothelium-dependent acetylcholine vasodilation [8], and do not affect dynamics of the posthemorrhagic disturbances in systemic and regional (hepatic) circulation. These data agree with our previous hypothesis that during the development of shock, low resistance of the cardiovascular system against circulatory hypoxia can be considered as dysregulatory pathology arising not due to primary damage to an organ, but resulting from the primary disturbances in the corresponding central control apparatus or in its local self-regulating system [1,4].

In conclusion, the use of cholinotropic agents with optimal profile of the receptor activity can be promising tool not only in the detailed examination of shock pathogenesis, but also in the development of pathogenetic treatment protocols.

REFERENCES

1. N. Ya. Kovalenko and D. D. Matsievskii, *Patogenez*, No. 2, 53-66 (2004).
2. N. Ya. Kovalenko and D. D. Matsievskii, *Byull. Eksp. Biol. Med.,* **140**, No. 8, 142-145 (2005).
3. A. B. Kosmachev, V. A. Belyaev, A. V. Khrabrova, *et al*., *Eksp. Klin. Farmakol*., **61**, No. 5, 3-5 (1998).
4. G. N. Kryzhanovsky, *Dysregulatory Pathology* [in Russian], Moscow (2002).
5. D. D. Matsievskii, *Byull. Eksp. Biol. Med.,* **138**, No. 10, 612-616 (2004).
6. D. D. Matsievskii, *Byull. Eksp. Biol. Med.,* **136**, No. 7, 115-118 (2003).

7. N. P. Podosinovikova, L. F. Gorobets, and V. B. Dolgo-Saburov, *Byull. Eksp. Biol. Med.,* **122**, No. 7, 75-77 (1996).
8. K. J. Broadly and D. B. Kelly, *Molecular*, No. 6, 142-193 (2001).
9. R. M. Eglen and R. L. Whiting, *J. Auton. Pharmacol*., **10**, No. 4, 233-245 (1990).
10. V. A. Pujol Lereis, F. J. Hita, M. D. Gobbi, *et al*., *Br. J. Pharmacol*., **147**, No. 5, 516-523 (2006).
11. H. Shi, H. Wang, and Z. Wang, *Mol. Pharmacol.*, **55**, No. 3, 497-507 (1999).
12. L. Walch, C. Brink, and X. Norel, *Therapie*, **56**, No. 3, 223-226 (2001).