Mechanisms of the Vasodilator Effect of Nitroglycerin and Chlorpromazine and Specificities of Their Interaction in a Model of Isolated Rat Thoracic Aorta

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The effect of nitroglycerin and chloropromazine (plegomazin) on norepinephrine-induced contraction of rat aorta rings is studied in experiments on the intact and deendothelized aorta, and the combined myolytic effect of the two preparations is assessed. A possible cross-tolerance between nitroglycerin and chloropromazine is studied in experiments simulating the development of vascular tolerance of nitroglycerin.

Key Words: *calcium ions; calmodulin; vasodilation; tolerance of nitrates; cross-tolerance*

Calmodulin and calcium ions (Ca^{2+}) play an important role in initiating the contraction of vascular smooth muscle cells. The cascade process of vasoconstriction comprises the following stages: 1) an increase of the intracellular concentration of free Ca^{2+}; 2) binding of 4 Ca^{2+} ions by calmodulin; 3) activation of kinase of light myosin chains by the Ca^{2+}-calmodulin complex; 4) myosin phosphorylation and stimulation of its ATPase-activity; 5) contraction of actin-myosin filaments in smooth muscle cells [11]. Two main pathways of pharmacological regulation of the activity of Ca^{2+}-calmodulin leading to myocyte relaxation and vasodilation are distinguished. The first of them is based on a change in the intracellular concentration of Ca^{2+}. Such a mechanism of myorelaxation is realized by Ca^{2+} channel blockers (nifedipine and verapamil) and organic nitrates, specifically, nitroglycerin (NG). After penetrating into the smooth muscle cell, NG undergoes enzymatic biotransformation, forming nitric oxide (NO). Interaction of NO with the heme group of the soluble fraction of guanylate cyclase leads to activation of the enzyme [3] and accumulation of cGMP, this being followed by a decrease in the intracellular calcium content [4] due to the blocking of Ca^{2+} entry into the cell [1,2,10] and activation of the mechanisms of its "entrapment" [8]. The second pathway is via direct inhibition of calmodulin. Such a mechanism underlies the vasorelaxing effect of phenothiazine derivatives (chlorpromazine - CP - and triphthazine), which, in therapeutic concentrations, selectively suppress calmodulin activity [6,13]. However, the existence of other possible mechanisms of NG- and CP-induced myorelaxation, as well as the presence of common components in the effect of preparations from both groups on smooth muscle tone, cannot be ruled out. Specifically, the role of endothelium in vasodilation induced by phenothiazine derivatives is not clear. In the literature available to us we have found no data characterizing the interaction between nitrates and calmodulin antagonists. On the other hand, a study of the combined application of NG and CP, which nave different mecha-

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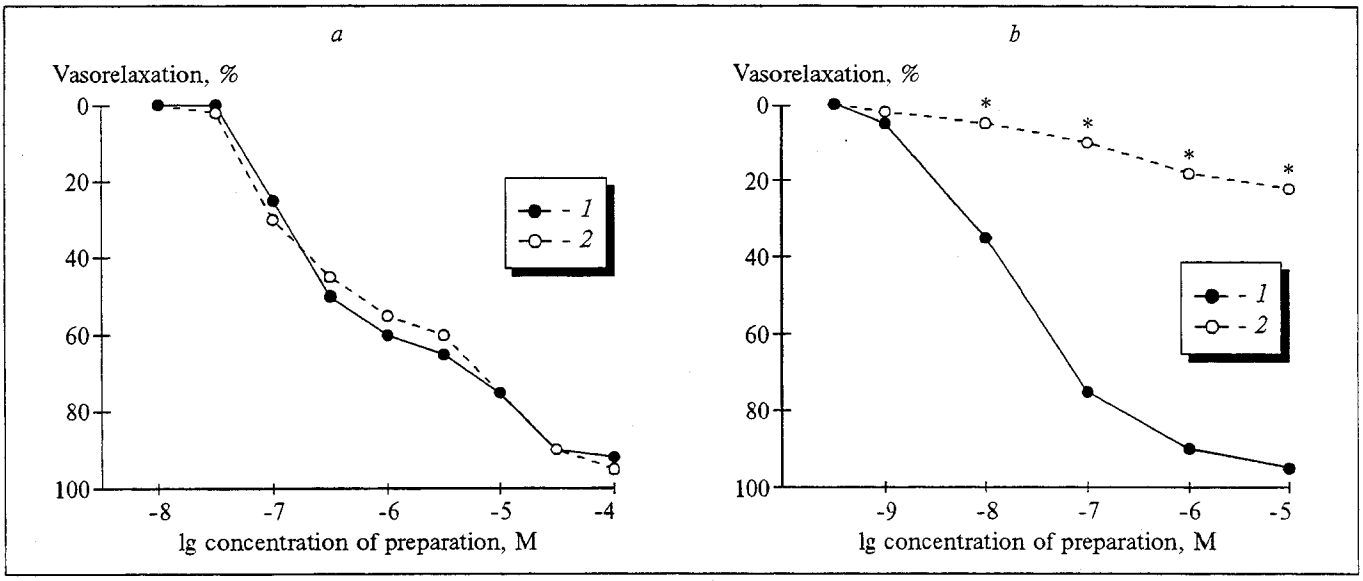

Fig. 1. Effect of NG (*a*) and CP (*b*) on relaxation of intact (*1*) and deendothelized (*2*) rat aorta segments. An asterisk denotes reliable differences from the control ($p<0.01$). Here and in Figs. 2 and 3 the results of 4–5 experiments are shown.

nisms of action but exert similar pharmacological effects on the vascular smooth muscle tone, is of indubitable interest to researchers.

MATERIALS AND METHODS

The experiments were carried out on male rats weighing 250-300 g. After the animals were killed under ether anesthesia, the thoracic aorta was isolated, carefully dissected from the surrounding tissues, and cut in segments [14]. A chain comprising 8 rings was placed in a bath for isolated organs with modified Krebs solution. The width of each ring was about 2 mm, and the length of the entire chain was 2.5 cm. The incubation temperature was 37°C; aeration was performed with carbogen. The initial loading was 2.0 g, and the equilibration period lasted 60 min. Contractions were caused by norepinephrine (NE) in a concentration of 10^{-6} M and recorded in the isometric mode on a Ugo-Basile apparatus (Italy). The effect of NG and CP on NE-induced contraction of isolated segments of rat thoracic aorta was studied by applying increasing concentrations of the preparations after the development of 2 stable responses to NE and the attainment of the plateau of the contractile response. The myorelaxing effect of the preparations was assessed using dose-effect curves and expressed in percent. The effects of NG and CP were compared by determining the effective concentration of the preparations (EC_{50}) which caused a 50% relaxation (*vs.* the maximum NE-induced contraction) of rat aorta segments, by the method of cumulative curves [7]. The aorta was deendothelized after Furchgott *et al.* [9]. In studies of the combined effect of NG and CP the myorelaxing effect of NG in the presence of threshold concentrations of CP was determined using the dose-effect curves.

In the series of experiments simulating the conditions of tolerance of NG isolated segments of the vessels were incubated for 60 min in a buffered Krebs solution (pH 7.2) or in a buffer containing 0.1 mM NG. After the end of incubation, segments were washed in fresh buffer for 5 min. After the equilibration and attainment of the plateau of the contractile response to 10^{-6} M NE, the NG- and CP-induced relaxation was studied.

The results were processed using methods of variational statistics and expressed as the mean and the standard error of the mean ($M\pm m$). The reliability of differences was assessed using Student's t test.

NE (Serva), Nitro (1% NG solution) (Orion, Finland), and Plegomazin (2.5% CP solution) (Gedeon Richter, Hungary) were used in the study.

RESULTS

As follows from the data presented in Fig. 1, NG and CP exhibited a manifest myorelaxing activity and were able to effectively suppress the spasmogenic effect of NE on segments of the rat thoracic aorta. The spasmolytic effect of NG and CP was dose-dependent and exponentially increased for application of the preparations in increasing concentrations. NG-induced myorelaxation had a reversible pattern: washing of aorta rings in a buffer solution entirely restored the sensitivity of smooth muscle cells to NE. On the other hand, incubation

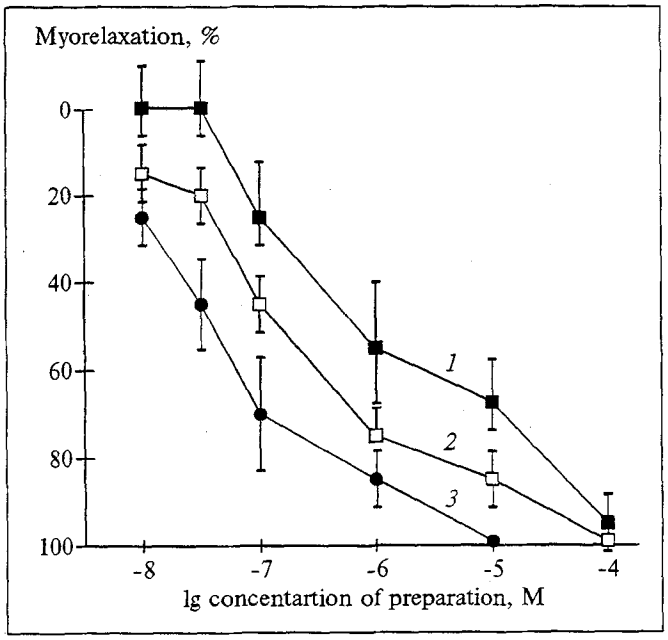

Fig. 2. Effect of CP on NG-induced relaxation of rat aorta. *1*) NG (control); *2*) NG with 10^{-10} M CP; *3*) NG with 10^{-9} M CP.

of aorta segments in the presence of high concentrations of CP (0.5-10 μM) led to a virtually irreversible myorelaxation. In this experimental model CP exhibited a higher vasorelaxing activity than NG: log EC_{50} for NG and CP was -6.55±0.21 and -8.34±0.32, respectively (n=5, p<0.05).

In another series of experiments we assessed the vasodilator effect of NG and CP on aorta segments with preliminarily destroyed endothelium. Deendothelization did not cause reliable changes in the myorelaxing activity of NG (Fig. 1, *a*). Meanwhile, the spasmolytic effect of CP was markedly reduced (Fig. 1, *b*). Relaxation did not exceed 25% of the control (an intact aorta), and was noted only in the presence of high doses of the preparation (0.5-10 μM). Thus, two components may be identified in the myorelaxing effect of CP: the effect of CP in a concentration from 10^{-9} to 10^{-6} M, which was endothelium-dependent in nature, and the vasodilator effect of CP in a concentration from 10^{-6} to 10^{-5} M, correlating with the inhibiting effect of the preparation on the calmodulin activity [5].

Our findings suggest that the leading component in the mechanism of the effect of CP is endothelium-dependent vasorelaxation. On the other hand, the vasodilator effect of NG and other nitric compounds capable of generating NO does not depend on the integrity of endothelium [3].

Figure 2 shows the results of the experiments where relaxation of aorta rings under the influence of increasing concentrations of NG was studied in the presence of 10^{-10} M CP. In this concentration CP alone exhibited no myorelaxing activity (the dose being lower than the threshold dose). When CP in this concentration was used in combination with NG, an effect of potentiation was observed (curve *2*). Combined application of increasing concentrations of NG and CP in a dose of 10^{-9} M led to a more marked relaxation of aorta rings (curve *3*).

Figure 3 presents the results of the experiments in which the vasorelaxing effect of NG and CP was assessed following incubation of aorta rings in a medium with a high content of NG. It was established that a 60-min incubation of vessels in a buffer containing 0.1 mM NG markedly weakens the myorelaxing effect of NG due to the development of tolerance for this preparation; in this case EC_{50} rose almost 120-fold as compared to the control values. Incubation of rat aorta rings with NG also markedly affected the indexes of CP-induced myorelaxation. Since CP-induced relaxation in aorta segments incubated with 0.1 mM NG did not exceed 20% of the control, we assessed the EC_{20} value: an increase of log EC_{20} from -8.35±0.32 to -4.27±0.24 (p<0.001) attested to the development of cross-tolerance between NG and CP (Fig. 3). The effect of incubation of aorta segments with CP on NG- and CP-induced relaxation was not assessed, since CP caused irreversible myorelaxation: the sensitivity of myocytes to NE could not be restored by washing in buffer solution.

The phenomenon of cross-tolerance between NG and CP corroborates our conclusion that CP-induced myorelaxation is endothelium-dependent in

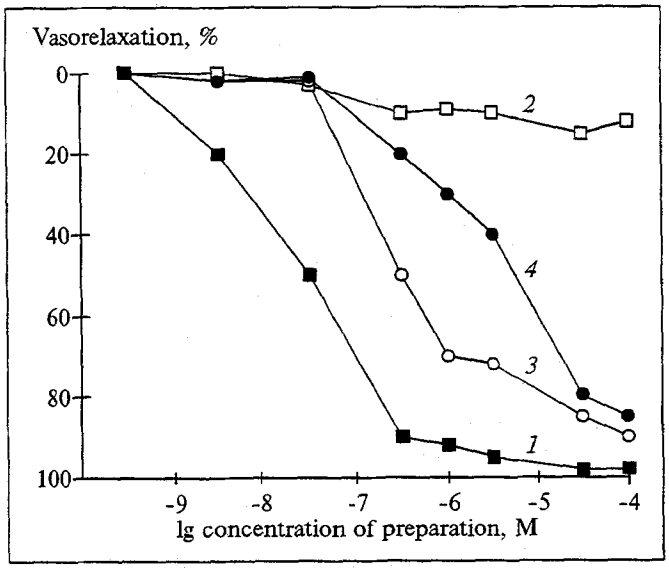

Fig. 3. Effect of incubation of rat aorta segments in medium with a high NG content on myorelaxing activity of CP (*1* and *2*) and NG (*3* and *4*). *1* and *3*) control (30-min incubation in a buffer); *2* and *4*) incubation in a buffer containing 0.1 mM NG.

nature. A similar endothelium-mediated mechanism of the vasodilator effect has also been described for endogenous biologically active substances: acetylcholine, serotonin, bradykinin, and histamine [9]. In addition, it has been shown that preliminary incubation with NG markedly reduces the sensitivity of vessels to acetylcholine [12].

REFERENCES

1. M. Yu. Men'shikov, P. V. Avdonin, E. V. Negresku, *et al., Byull. Vsesoyuz. Cardiol. Nauch. Tsentra,* № 2, 25-30 (1986).
2. P. V. Sergeev, G. I. Storozhakov, A. S. Dukhanin, *et al., Byull. Eksp. Biol. Med.*, **114**, № 11, 487-489 (1992).
3. G. I. Storozhakov, P. V. Sergeev, V. Yu. Shilo, and A. S. Dukhanin, *Khim.-Farm. Zh.,* № 11-12, 4-10 (1992).
4. V. Yu. Shilo, G. I. Storozhakov, P. V. Sergeev, *et al., Byull. Eksp. Biol. Med.*, **113**, № 3, 292-293 (1992).
5. M. Asano, *J. Pharmacol. Exp. Ther.*, **251**, 764 (1989).
6. M. Asano, Y. Suzuki, and M. Hidaka, *Ibid.*, **220**, 191-196 (1982).
7. A. Fakstorp and J. Pedersen, *Acta Pharmacol. (Kbh.),* **13**, 359-367 (1957).
8. K. Furokawa and H. Nakamura, *J. Biochem. (Tokyo),* **101**, 287-290 (1987).
9. R. Furchgott and J. Zawadski, *Nature,* **288**, 373-376 (1980).
10. T. Godfraind, *Europ. J. Pharmacol.,* **126**, 341-343 (1986).
11. T. Itoh, *Jap. J. Pharmacol.,* **55**, 1-9 (1991).
12. M. E. Ljusegren, J. Ahlner, and K. L. Axelsson, *Pharmacol. Toxicol.,* **62**, 302-307 (1988).
13. N. Ogata and T. Narahashi, *J. Pharmacol. Exp. Ther.,* **252**, 1142-1149 (1990).
14. J. C. Wanstall and S. R. O'Donnel, *Proc. Austral. Physiol. Pharmacol. Soc.,* **16**, 49 (1985).

Ammonium Succinate Is an Effective Corrector of Cerebral Circulatory Hypoxia

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It is demonstrated that ammonium succinate is capable of increasing the survival of rats with acute brain ischemia. In transient brain ischemia therapeutic injection of ammonium succinate prevents the development of postischemic hypoperfusion and hypooxygenation of the brain. The antiischemic effect of ammonium succinate is due to a decrease of the oxygen affinity of hemoglobin and to limitation of the accumulation of malonic dialdehyde, a secondary product of lipid peroxidation, in the brain. No vasotropic activity of ammonium succinate is revealed.

Key Words: *brain ischemia; brain circulation; oxygen metabolism in the brain; ammonium succinate*

Antihypoxants are widely used as correctors of disturbances of the cerebral circulation due to the leading role of hypoxia in the pathogenesis of cerebrovascular disorders. Antihypoxants, which optimize the metabolic processes without exerting any of the possible, frequently unpredictable, adverse vasodilator effects [3], make up a promising group of antiischemic agents. The succinic acid (SA) derivatives exhibit a pronounced antihypoxic activity [4,8]. In view of the foregoing, we studied the antiischemic

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