Effects of Verapamil and Amiodarone on Sympathoadrenal System and Balance of Excitatory and Inhibitory Amino Acids in Rat Medulla Oblongata

A. Yu. Turovaya, P. A. Galenko-Yaroshevskii, A. Kh. Kade, A. E. Uvarov, M. I. Kiguradze*, N. G. Khvitiya*, and D. R. Tatulashvili*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 6, pp. 632-634, June, 2005
Original article submitted November 22, 2004

Local injection of verapamil into ventrolateral region of the medulla oblongata triggered the release of epinephrine. Verapamil increased the total content of norepinephrine and epinephrine by 560% and decreased the content of serotonin by 46%. Verapamil had no effect on norepinephrine/epinephrine and norepinephrine/(norepinephrine+epinephrine) ratios in normal rats. Blockade of K^+-channels in the medulla oblongata by local injection of 0.001 mg amiodarone did not change the levels of epinephrine and norepinephrine and norepinephrine/epinephrine and norepinephrine/(norepinephrine+epinephrine) ratios. In the medulla oblongata, verapamil proportionally increased the levels of norepinephrine, dopamine, and L-DOPA. Similarly, amiodarone increased the levels of L-DOPA and dopamine by 2.6 and 3.2 times, respectively. Amiodarone shifted the ratio of neuroactive amino acids towards inhibitory transmitters.

Key Words: *verapamil; amiodarone; amino acids; dopaminergic regulation; sympathoadrenal system*

Activation of the sympathetic autonomic nervous system plays an important role in the pathogenesis of arterial hypertension [14]. The bulbar vasomotor center is a source of neurogenic vascular tone. It participates in the formation of vasomotor reflexes and in respiratory-circulatory coordination [8]. It was hypothesized that abnormal hyperactivity of the bulbar vasomotor center during arterial hypertension is associated with activation of adrenergic processes in the brainstem [13]. Bilateral destruction of a small region in ventrolateral surface of the medulla oblongata decreased arterial pressure; this effect is comparable with hypotension caused by proximal ligation of the spinal cord [3]. Calcium antagonists exert antihypertensive, spasmolytic, and coronarolytic effects [9,10, 12]. Amiodarone inhibits activation of α- and β-adrenoceptors in the heart via blockade of K^+-channels and, to a lesser degree, Na^+- and Ca^{2+}-channels of cardiomyocyte membrane. Sympatholytic activity and the blockade of potassium and calcium channels with amiodarone prolong action potential and decrease of its amplitude, which reduces oxygen demand of the myocardium [7]. Our aim was to study the effect of amiodarone, a calcium antagonist and predominant blocker of potassium channels, on sympathoadrenal, serotoninergic, and GABAergic systems in the medulla oblongata, and on the content of inhibitory and excitatory amino acids after single injection of amiodarone into the medulla oblongata.

MATERIALS AND METHODS

The study was carried out on male rats (n=21) weighing 180-200 g. The rats were randomly divided into

Krasnodar Regional Medical Research Center; N. V. Karsanov Republican Research Center for Medical Biophysics and New Biomedical Technologies, Tbilisi

0007-4888/05/1396-0665

control and two experimental groups (7 rats in each group). The animals were maintained under vivarium conditions on the standard ration and unrestricted water. Verapamil and amiodarone were injected into the medulla oblongata in experimental groups 1 and 2, respectively.

The rats were fixed in a stereotaxic apparatus. The rostral ventrolateral surface of the medulla oblongata was scanned with 1 mm resolution from the middle of pair XII roots (zero level) to 6 mm in the rostral direction, to 3-4 mm into lateral region of medial line, and to the depth of 4 mm from the ventrolateral surface of the medulla oblongata. In experimental groups, locally anesthetized rats were injected with verapamil (0.025 μg) or amiodarone (0.001 mg) [5,6,11]. The injections were performed with a Hamilton microsyringe bilaterally at 0.4 mm rostrally from zero level. Experimental rats demonstrated normal mean blood pressure (104.6±2.7 mm Hg). Control rats were similarly injected with artificial liquor containing (in mM): 150 Na^+, 3.0 K^+, 1.4 Ca^{2+}, 0.8 Mg^{2+}, 31.0 PO_4^{2-}, 155 Cl^-, pH 7.4) [1]. The rats were decapitated, the medulla oblongata was isolated and placed into liquid nitrogen. Then it was minced on ice to prepare the homogenate. Catecholamines, serotonin, and neuroactive amino acids were assayed spectrofluorimetrically in the homogenate [2,4].

Neuroactive amino acids were measured by reversed phase chromatography on a 3×150 mm column (Diasorb-130C_{16}T, 8 μl, Elsico). Isocratic elution was performed after precolumn derivatization with o-phthalaldehyde and 2-mercaptoethanol. Spectrofluorimetry was carried out at λ_{excit}=338 nm and λ_{emiss}=425 nm. The data were processed statistically using STAT Soft software and Student's *t* test at $p<0.05$.

RESULTS

A single local injection of verapamil into the ventrolateral region of the medulla oblongata triggered the release of epinephrine and increased the levels of epinephrine and norepinephrine by 505 and 519%, respectively. The total content of norepinephrine and epinephrine increased by 560% (Table 1). Despite pronounced increase in the absolute content of catecholamines under the action of verapamil, the ratios of norepinephrine/epinephrine and norepinephrine/(norepinephrine+epinephrine) in normal rats remained unchanged. It can be hypothesized that sharp elevation of epinephrine and norepinephrine contents in the medulla oblongata in verapamil-treated rats is a compensatory reaction and a mechanism of rapid adaptation to calcium deficiency, which produces no imbalance in the sympathoadrenal system. Predominant blockade of potassium channels in the medulla oblongata by local injection of amiodarone (0.001 mg) produced no significant changes in epinephrine and norepinephrine contents. In addition, the total content of epinephrine and norepinephrine, and the regulatory ratios of norepinephrine/epinephrine and norepinephrine/(norepi-

TABLE 1. Effect of Verapamil and Amiodarone on Content of Epinephrine, Norepinephrine, Dopamine, L-DOPA, and Serotonin and on Ratio of Excitatory and Inhibitory Amino Acids in Rat Medulla Oblongata (*M*±*m*)

Parameter	Group		
	control	verapamil	amiodarone
Epinephrine, nmol/mg protein	3.75±0.62	22.7±4.8*	4.61±0.82
Norepinephrine, nmol/mg protein	5.2±1.1	32.2±4.9*	8.57±1.93
Dopamine, nmol/mg protein	3.6±0.8	23.7±7.2*	11.5±2.5*
L-DOPA, nmol/mg protein	4.16±0.21	26.0±3.2*	12.0±2.1*
Serotonin, nmol/mg protein	0.05±0.01	0.027±0.009*	0.020±0.008*
Norepinephrine+epinephrine	8.95±1.68	59.1±13.7*	11.7±2.3
Norepinephrine/epinephrine	1.38±0.01	1.6±0.3	1.07±0.33
Norepinephrine/(norepinephrine+epinephrine)	0.57±0.03	0.57±0.04	0.59±0.04
Dopamine/(norepinephrine+epinephrine)	0.38±0.01	0.34±0.06	0.90±0.15*
Aspartate, μmol/g wet tissue	2.88±0.31	1.57±0.16*	4.37±0.45*
Glutamate, μmol/g wet tissue	7.1±0.5	3.53±0.41*	4.47±0.34*
Glycine, μmol/g wet tissue	1.22±0.16	3.51±0.53*	3.51±0.53*
GABA, μmol/g wet tissue	3.4±0.2	3.0±0.2	8.51±0.41*
(Aspartate+glutamate)/(glycine+GABA)	2.21±0.22	0.74±0.10*	0.59±0.06*

Note. *$p<0.05$ compared to the control.

nephrine+epinephrine) did not change. Thus, amiodarone had no significant effect on activity of the sympathoadrenal system.

The level of norepinephrine precursor dopamine increased by 558% in parallel to accumulation of norepinephrine. Similar increase (by 474%) was observed for the content of L-DOPA, which is converted to dopamine in the reaction catalyzed by dopamine hydroxylase. Injection of verapamil into the medulla oblongata increased activity of the sympathoadrenal and dopaminergic systems. Verapamil did not change the dopamine/(norepinephrine+epinephrine) ratio. By contrast, it decreased the level of serotonin by 46%. In contrast to lipophilic β-adrenoblockers and calcium antagonists crossing the blood-brain barrier, amiodarone produced no significant shift in dopaminergic regulation: it elevated the contents of L-DOPA and dopamine by 2.6 and 3.2 times, respectively. The absence of significant increase in norepinephrine level attests to disturbances in the mechanism of dopamine conversion into norepinephrine. Amiodarone produced a shift in favor of dopaminergic regulation: it increased the dopamine/(norepinephrine+epinephrine) ratio by 2.4 times. In addition, amiodarone markedly decreased serotonin content (Table 1), which attests to inhibition of serotoninergic regulation.

Strengthening of the sympathoadrenal regulation was accompanied by a decrease in the levels of aspartate and glutamate by 45 and 50%, respectively. The content of glycine increased by 65%, while that of GABA did not significantly change (Table 1). In the medulla oblongata, verapamil shifted the ratio between excitatory and inhibitory amino acids towards inhibitory transmitters. Injection of amiodarone into the medulla oblongata increased the content of aspartate by 52% and decreased the level of glutamate by 63%. As a result, the total content of excitatory amino acids was similar in the experimental and control groups. Amiodarone increased the contents of glycine and GABA (inhibitory amino acids) by 188 and 150%, respectively (Table 1). This effect modulated the balance in the system of neuroactive amino acids towards inhibitory transmitters.

These findings improve understanding of the mechanisms underlying disturbances in permeability of the blood-brain barrier during ischemia. In addition, they demonstrate a possibility of correcting these processes with verapamil and amiodarone.

REFERENCES

1. I. I. Afanas'ev, M. L. Dvorkina, I. A. Novoselov, and K. S. Raevskii, *Eksp. Klin. Farmakol.*, No. 1, 3-7 (2003).
2. V. I. Kocherga and I. L. Opentanova, *Vopr. Med. Khim*., No. 1, 81-85 (1980).
3. V. P. Lebedev, in: *Physiology of Circulation. Control of Blood Flow* [in Russian], Leningrad (1986), p. 295.
4. Yu. E. Razvodovskii, E. M. Doroshenko, and M. M. Selevich, *Eksp. Klin. Farmakol.*, No. 1, 12-16 (2003).
5. A. Yu. Turovaya, A. Kh. Kade, A. P. Galenko-Yaroshevskii, *et al.*, *Byull. Eksp. Biol. Med.*, Suppl. **2**, 89-93 (2001).
6. A. Yu. Turovaya, A. Kh. Kade, P. A. Galenko-Yaroshevskii, *et al.*, *Ibid.*, Suppl. **2**, 63-65 (2002).
7. C. Binggeli, R. Corti, I. Sudano, *et al.*, *Hypertension*, **39**, 892 (2002).
8. K. Ezure, Y. Oku, and I. Tanaka, *Brain Res.*, **632**, 216-224 (1993).
9. J. A. Ferrendelli, in: *Calcium Regulation by Calcium Antagonists*, Washington (1982), p. 143.
10. A. Hayar and P. G. Guyenet, *Am. J. Physiol. Heart Circ. Physiol.*, **277**, H1069-H1080 (1999).
11. S. Ito, F. J. Gordon, and A. F. Sved, *Am. J. Physiol. Integr. Comp. Physiol.*, **276**, R1600-R1607 (1999).
12. M. T. Kailasam, R. J. Parmer, J. H. Cervenka, *et al.*, *Hypertension*, **26**, 143-149 (1995).
13. S. Z. Lander, *Biochem. Pharmacol.*, **23**, 1793-1800 (1974).
14. K. Y. Rahn, M. Barenbrock, and M. Hausberg, *J. Hypertens.*, **17**, Suppl. 3, S11-S14 (2000).
15. J. Tank, A. Diedrich, C. Schroeder, *et al.*, *Hypertension*, **38**, 1377-1381 (2001).