Oxygen-Dependent Mechanisms Underlying the Antiischemic Effect of Verapamil and Amlodipine

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Verapamil and amlodipine produced a potent antiischemic effect and reduced the area of myocardial infarction in rats. The observed changes were accompanied by inhibition of lipid peroxidation. In contrast to verapamil, amlodipine in a dose of 50 ng/ml *in vitro* decreased hemoglobin affinity for oxygen. Moreover, amlodipine in a concentration of 2 ng/ml decreased the content of malonic dialdehyde and activity of superoxide dismutase in the blood.

Key Words: *antiischemic effect; hemoglobin affinity for oxygen; lipid peroxidation; verapamil; amlodipine*

The mechanisms underlying antiischemic activity of calcium channel blockers were extensively studied [8,9]. However, little is known about the influence of these preparations on hemoglobin affinity for oxygen (HAO). The decrease in HAO considerably promoted oxygen release from ischemic myocardium under conditions of limited coronary reserves. Calcium channel blockers produce various and even opposite changes in HAO. Previous studies showed that verapamil *in vitro* prevents deoxygenation of the blood [2]. Another calcium channel blocker cerebrocrust belonging to the group of 1,4-dihydropyridine derivatives decreases HAO and improves oxygen-transporting function of the blood [4]. Under certain conditions the decrease in HAO is accompanied by activation of lipid peroxidation (LPO). This is one of the major mechanisms underlying ischemic injury in cell membranes. It is important to evaluate the effects of verapamil and amlodipine on HAO and LPO.

MATERIALS AND METHODS

The area of myocardial infarction in rats was determined gravimetrically and expressed in percents of the weight of the left ventricle. The study was performed 4 h after ligation of the anterior descending branch of the left coronary artery at the level of the lower edge of the left auricle.

HAO was studied in blood samples taken from the right auricle of rabbit heart. Verapamil (80 and 400 ng/ml) and amlodipine (10 and 50 ng/ml) were dissolved in 0.1 ml physiological saline and added to 5 ml heparinized blood. Blood samples were incubated at 37°C for 120 min. HAO was determined by P_{50} after mixing [1]. Two portions of the sample (1.5-2.0 ml) were exposed to maximum oxygenation and deoxygenation. P_{50} was assayed in 2 equal samples on an ABL-330 Radiometer gas microanalyzer.

Malonic dialdehyde (MDA) content in the plasma from rats (200-250 g) was measured in the initial state and 4 h after coronary artery ligation [5]. Fe^{2+}-induced chemiluminescence (CL) in plasma samples was recorded on a BKhL-06 biochemiluminometer. Verapamil (1 and 5 mg/kg) and amlodipine (0.25 and 1.00 mg/kg) were injected intravenously before coronary artery ligation.

In *in vitro* experiments on donor blood hemolysate accumulation of MDA and activity of Cu,Zn-superoxide dismutase (SOD) were studied. The amount of SOD decreasing the rate of cytochrome *c* reduction in the xanthine-xanthine oxidase system by 50% was taken as a unit of enzyme activity [7]. MDA content was estimated by the amount of thiobarbituric acid-

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TABLE 1. Effects of Verapamil and Amlodipine on the Area of Necrosis and Intensity of LPO in Rats with Experimental Myocardial Infarction (*M*±*m*)

Series	Area of myocardial infarction, % of the weight of the left ventricle	MDA, nmol/ml		Fe^{2+}-induced CL, arb. units	
		initial level	4 h	initial level	4 h
Control (*n*=10)	47.38±0.45	0.405±0.006	0.644±0.011	0.450±0.006	0.841±0.014
Verapamil					
1 mg/kg (*n*=16)	40.59±0.56*	0.393±0.026	0.596±0.009*	0.431±0.019	0.762±.004*
5 mg/kg (*n*=16)	33.58±0.79*	0.4020±0.0045	0.523±0.008*	0.445±0.005	0.708±0.008*
Amlodipine					
0.25 mg/kg (*n*=16)	38.53±0.60*	0.398±0.009	0.599±0.008*	0.444±0.006	0.808±0.007
1.0 mg/kg (*n*=16)	33.69±1.22*	0.399±0.006	0.537±0.013*	0.439±0.008	0.860±0.011

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

reactive substances [6]. $FeSO_4$ (1.20 μM) and NADPH (0.77 μM) were used to activate NADPH-dependent LPO [10]. Changes in MDA content and SOD activity were recorded 5 and 120 min after administration of the preparation.

The results were analyzed by Student's *t* test.

RESULTS

Verapamil and amlodipine dose-dependently reduced the area of experimental myocardial infarction in rats. MDA accumulation in the plasma was less pronounced in rats receiving the test preparations (Table 1).

In rats treated with verapamil and amlodipine the intensity of Fe^{2+}-induced CL decreased less significantly than in control animals. In contrast to verapamil, increasing the dose of amlodipine did not potentiate this effect (Table 1).

Verapamil in a dose of 80 ng/ml *in vitro* increased HAO. Values of P_{50} in verapamil-treated and control rats were 26.02±0.52 and 30.19±0.50 mm Hg, respectively. Increasing the dose of verapamil did not potentiate this effect. It should be emphasized that amlodipine in a dose of 50 ng/ml markedly increased P_{50}. MDA content increased after incubation with 80 ng/ml verapamil for 5 min. However, amlodipine in a dose of 2 ng/ml inhibited MDA accumulation (Table 2).

TABLE 2. Effects of Verapamil and Amlodipine on HAO (*M*±*m*)

Series		Concentration, ng/ml	P_{50}, mm Hg
Control	(*n*=8)		30.19±0.50
Verapamil	(*n*=7)	80	26.02±0.52*
	(*n*=7)	400	27.33±0.34*
Amlodipine	(*n*=7)	10	29.42±0.37
	(*n*=6)	50	32.68±0.77*

Two-hour incubation with verapamil decreased SOD activity in the blood, but had no effect on MDA accumulation (Table 2). Incubation with amlodipine in a dose of 2 ng/ml for 5 and 120 min inhibited MDA accumulation (Table 3). Changes in SOD activity were observed only after 2-h incubation with amlodipine.

Our findings suggest that the increase in HAO produced *in vitro* by verapamil contributes to inhibition of LPO in rats with experimental myocardial infarction. Similar results were obtained in experiments on rabbits with hyperthermia. Our assumption is confirmed by published data that verapamil prevents de-

TABLE 3. Effects of Verapamil and Amlodipine on MDA Accumulation and SOD Activity in the Blood (*M*±*m*, *n*=10)

Series	Concentration, ng/ml	MDA, nmol/g hemoglobin		SOD, U/g hemoglobin	
		5 min	120 min	5 min	120 min
Control		4.00±0.07	3.10±0.07	1281±33	1281±32
Verapamil	16.0	4.00±0.11	2.80±0.12	1281±24	931±45*
	80.0	6.00±0.11*	2.90±0.07	1276±54	1077±54*
Amlodipine	0.4	4.00±0.11	3.00±0.13	1282±32	1207±20
	2.0	2.30±0.11*	2.40±0.12*	1284±26	404±64*

oxygenation of the blood and decreases the intensity of Fe^{2+}-induced CL [2,3].

Amlodipine decreased HAO (50 ng/ml), MDA content, and SOD activity (2 ng/ml). These changes probably underlie the antiischemic effect of this preparation.

In vitro experiments showed that the decrease in HAO produced by amlodipine was not accompanied by activation of LPO. Moreover, amlodipine reduced the area of experimental myocardial infarction and abolished MDA accumulation in rat plasma 4 h after coronary artery ligation.

These data amplify modern notions of the mechanism underlying antiischemic activity of amlodipine. Our findings should be taken into account when evaluating the effects of verapamil during pharmacotherapy of patients with arterial hypertension and coronary heart disease.

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