PHARMACOLOGY

Effect of Cytochrome *c* on the Necrosis Zone in Transitory Myocardial Ischemia

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The cardioprotector effect of cytochrome *c* during a 15-min complete blocking of the anterior descending branch of the left coronary artery was studied in rat experiments. Cytochrome *c* in a dose of 20 mg/kg was found to noticeably reduce the necrosis zone 4 h after transitory ischemia. The protective effect of a single injection of cytochrome *c* was virtually undetectable after 72 h, this pointing to the need for a course of treatment.

Key Words: *myocardial ischemia; cytochrome c; injury-slowing effect*

The importance of studying the effects of antiischemic agents in coronary spasm under conditions of subsequent reperfusion makes investigations of the cardioprotector action of compounds of this type a priority in experiments with simulation of transitory myocardial ischemia. Moreover, the idea of a possible transitory slow-down of myocardial infarction formation under the effect of antiischemic agents ("injury-slowing interventions"), which does not provide for a stable protective effect [6], prompts a study of the time course of ischemic and myocardial necrosis zones at later periods, 24-72 h after the reproduction of temporary occlusion of the coronary arteries.

In the present research we compared the cardioprotector effects of biotechnological cytochrome *c* under conditions of permanent occlusion of the coronary artery and in transitory myocardial ischemia and studied the efficacy of a course of cytochrome *c* therapy in ischemic alteration of the myocardium.

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MATERIALS AND METHODS

A 15-min transitory myocardial ischemia was used in the experiments, as this was previously discovered to produce a stable injurious effect [2]. Transitory coronary insufficiency was induced in white outbred male rats weighing 220-300 g by temporary ligation of the descending branch of the left coronary artery at the level of the lower edge of the left atrial auricle. The ligature was placed under the coronary artery under ethaminal narcosis (40 mg/kg intraperitoneally) and artificial ventilation of the lungs. Sixty-one animals were used in the study. Necrotic and ischemic zones were assessed 4 and 72 h after coronary artery occlusion by the differential indicator method [3,5]. Biotechnological cytochrome *c* was prepared at the Biochemistry Department of the Research Institute of Protein Synthesis (headed by E. R. Davidov). A previous comparative pharmacological study showed a virtually identical biological activity of cytochrome *c* of animal origin and its biotechnological analog [1,4]. In studies of the effect of cytochrome *c* on the size of the necrotic zone in transitory ischemia the drug was injected intravenously in doses of 5

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TABLE 1. Effect of Cytochrome *c* on the Size of Necrotic and Ischemic Zones in Permanent 4-Hour Occlusion (I) and Transitory Myocardial Ischemia 4 (II) and 72 (III) h after Coronary Artery Occlusion

Experimental series	Dose, mg/kg	*n*	Ischemic zone, % of total myocardial mass	Necrosis zone, % of total myocardial mass	Necrosis zone, % of ischemic zone
I. Control		17	34.0±2.6	22.0±2.0	68.0±4.3
Cytochrome *c*	20.0	7	27.0±2.9	9.0±1.4*	32.0±3.4*
	5.0	6	38.0±3.0	26.0±2.6	64.0±3.4
II. Control		13	29.8±2.2	13.5±2.1	45.8±6.9
a) Cytochrome *c*	20.0	7	25.4±0.9	6.2±1.2	24.9±5.9*
	5.0	6	26.9±3.0	11.8±2.8	42.9±7.3
b) Cytochrome *c*	20.0	8	34.4±3.2	8.1±1.8	24.0±4.8*
III. Control		11	27.2±2.4	11.5±2.3	41.1±8.6
Cytochrome *c* (course of injections)	20.0	6	27.5±2.0	4.2±2.0	15.0±4.5*
a) Cytochrome *c* (single injection)	20.0	10	25.7±0.9	12.2±2.3	47.5±8.9

Note. Asterisk shows difference vs. control at $p<0.05$; a) cytochrome *c* injection at 5th min of reperfusion; b) cytochrome *c* injection 10 min before ligature.

and 20 mg/kg at the 5th min of reperfusion and in a dose of 20 mg/kg 10 min before coronary artery ligature. For assessing the effect of cytochrome *c* on the necrotic zone 72 h after transitory myocardial ischemia the drug was intravenously injected in a dose of 20 mg/kg at the 5th min of reperfusion and then intraperitoneally twice daily for 3 days before the size of the myocardial infarction zone was determined. In the control cytochrome *c* activity was examined after a single injection at the 5th min after ligature with assessment of infarction size after 72 h. The results were statistically processed using Student's *t* test.

RESULTS

Previously it was revealed that during permanent occlusion of the coronary artery cytochrome *c* infused intravenously in a dose of 20 mg/kg 5 min after ligature reduced the necrosis zone almost twofold, causing no noticeable shifts of the ischemic zone [1,4]. The effect of a dose of 20 mg/kg on the size of the infarction zone in transitory ischemia was found to be similar to the effect of the enzyme for a permanent 4-hour occlusion of the coronary artery. As is seen from the data presented in Table 1 (IIb), cytochrome *c* injected 10 min before ligature reliably decreased the size of the necrotic zone, which was 24.0±4.8% of the ischemic zone vs. 45.8±4.3% in the control. A similar cardioprotector effect was observed when the drug was injected in an analogous dose at the 5th min of reperfusion. If the cytochrome *c* dose was reduced to 5 mg/kg its antiischemic effect virtually disappeared.

Hence, cytochrome *c* in a dose of 20 mg/kg had a manifest cardioprotector effect both if injected before ligature and at the 5th min after reperfusion.

It is noteworthy that cytochrome *c* injected at the 5th min of reperfusion significantly reduced the incidence of postocclusion rhythm disorders (64% in the control vs. 17% in the experimental series).

During assessment of the size of myocardial infarction 72 h after transitory ischemia, the drug (injected in a course of therapy) had a manifest cardioprotector effect by sharply reducing the necrotic zone (to 15.0±4.5% of the ischemic zone vs. 41.1±8.6% in the control).

Analysis of the time course of reduction of the necrotic zone indicates a higher therapeutic effect of cytochrome *c* administered in a course of injections. A single injection of the drug at the 5th min of reperfusion had virtually no effect on the extent of ischemic involvement 72 h later, whereas 4 h after transitory ischemia a manifest protective effect of the agent was observed. These data confirm that the limitation of the necrosis zone 4 h after drug injection does not lead to a stable reduction of the infarction zone.

The "injury-slowing interventions" phenomenon dictated the need to reinject cytochrome *c* to fortify the protector effect on the ischemic myocardium in the zone bordering the infarction area.

The results clearly demonstrate the promise of clinical trials of cytochrome *c* as an agent mitigating ischemic alteration both in acute myocardial infarction and in transitory coronary insufficiency.

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Effect of Aminooxyacetic Acid on the Antidote Activity of Diazepam Under the Action of GABA-Lytics

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Pretreatment of mice with aminooxyacetic acid enhances the antidote activity of diazepam against picrotoxin but not bicuculline. It is claimed that GABA-transaminase inhibitors may be promising candidates as an antidote in complex therapy of seizures induced by GABA-lytics blocking the chloride ionophore.

Key Words: *aminooxyacetic acid; diazepam; picrotoxin; bicuculline; toxicity*

It is known that application of anticonvulsants in combination potentiates their activity [2]. For example, the antidote activity of 1,4-benzodiazepines against GABA-lytica is enhanced by barbiturates [9], glycine [8], and aminooxyacetic acid (AOAA), which inhibits GABA-transaminase [7]. Clonazepam, injected into rats after their pretreatment with AOAA was found to have a less pronounced anticonvulsant activity in picrotoxin intoxication [7]. The effect of AOAA on the antidote efficacy of diazepam in intoxication induced by GABA-lytics has not beeen assessed, nor have the effects of AOAA on the functional state of the benzodiazepine and muscarinic receptors been examined. It is known that modulation of the M-cholinergic receptors by cholinotropic compounds alters the sensitivity of animals to picrotoxin [4].

In this study we examined the antidote activity of diazepam against the background of AOAA upon intoxication of albino mice with bicuculline or picrotoxin and assessed the effect of AOAA on the specific binding of the ligand of muscarinic receptor with synaptic membranes isolated from the brain of intact mice.

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MATERIALS AND METHODS

Experiments were performed on male albino mice weighing 25-30 g. Picrotoxin and bicuculline were suspended in normal saline with Tween-80. Aminooxyacetic acid was dissolved in normal saline and neutralized with sodium bicarbonate. The toxicity of the GABA-lytics was evaluated in probit analysis 2 h after injection of AOAA in a dose of 50 mg/kg. Diazepam (5 mg/kg) was injected 10 min prior to the toxins. All compounds used in this study were from Sigma. The preparations were injected intraperitoneally (0.2 ml solution per 10 g body weight). The effect of AOAA (10^{-10}-10^{-5} M) on the specific binding of [N-methyl-^{3}H]methyl-

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