Long-Term Cardiac Protective Effect of Nitric Oxide

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We have examined the effect of the dinitrosyl iron complexes, the NO donors, on the resistance of isolated heart to ischemia and reperfusion, as well as the effect of quercetin, an inhibitor of HSP70 transcription, on NO-induced enhancement of cardiac resistance to ischemia and reperfusion. The iron complexes were found to produce dose- and time-dependent protective effect and enhance the resistance of isolated heart to rhythm and contraction disorders during reperfusion. The maximum protective effect was observed 12-24 h postinjection of the 200 mg/kg dose. Quercetin completely prevented protective effect of the NO donor. The property of NO donors to stimulate HSP70 synthesis and the present data indicate that NO-dependent activation of HSP70 synthesis is a natural mechanism of cardiac protection against disturbances provoked by ischemia and reperfusion.

Key Words: *heart; nitric oxide; HSP70*

Nitric oxide (NO) donors were shown to enhance cardiac resistance to ischemia and reperfusion [1]. We assessed the development of protective effect immediately after injection of NO donor or during its infusion. It is supposed that the fast protective effect of NO donors is related to coronary blood flow increase and to decrease aggregation of neutrophils [1]. Previously we showed that the NO donor, dinitrosyl iron complex (DNIC) induces the synthesis of heat shock proteins (HSP70) [6]. Maximum accumulation of HSP70 was observed 24 h postinjection of DNIC. Based on these data and taking into account an important role of HSP70 in the anti-ischemic cardiac protection [2,5], we supposed that an NO donor could induce a more prolong effect, which could be related to HSP70.

Our aim was to test the hypothesis on the important role of NO-dependent activation of HSP70 synthesis in the long-term protective effect of NO donors. To this end we studied: 1) dynamics of NO in blood and heart after injection of NO donor; 2) dynamics of isolated heart resistance to ischemia and reperfusion after injection of NO donor; and 3) effect of quercetin, an inhibitor of HSP transcription, on the NO-induced protective effect.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 250-300 g. NO donor was DNIC 1:2 [8], which is transformed in the organism into paramagnetic DNIC 1:20 [7]. The organ and tissue levels of DNIC 1:20 can be determined by electron paramagnetic resonance (EPR) [7].

DNIC 1:2 was injected into the tail vein in doses of 100, 200, and 300 mg/kg. The animals were decapitated 1, 12, and 24 h postinjection of DNIC 1:2, and DNIC 1:20 was determined in blood and heart by EPR.

Quercetin (Sigma) was used as HSP70 transcription inhibitor [4]. Quercetin was injected intraperitoneally (5 mg/kg) 30 min prior injection of DNIC 1:2.

Ischemic and reperfusion damage was simulated on the hearts isolated by Langendorff [3]: coronary blood flow was totally arrested for 15 min, thereafter

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perfusion was restored, and observations were made during the next 15 min of reperfusion. Damage to the heart was assessed by the rhythm and contraction disturbances [2]. Significance of differences between the groups was analyzed using Student's *t* test.

RESULTS

Injection of DNIC 1:2 is accompanied by time- and dose-dependent increase in blood and cardiac DNIC 1:20 (Table 1). The content of DNIC 1:20 did not differ from the initial zero level 12 h after injection of DNIC 1:2.

Hearts of control rats and test rats treated with NO donor respond to total ischemia and reperfusion (Fig. 1). Ischemia led to a marked decrease in contractile amplitude in all animals. During reperfusion, the contractile amplitude in the control group was 8% of the initial value. In addition, in 70% control rats reperfusion resulted in fibrillation and cardiac arrest. Injection of DNIC (100 mg/kg) did not affect contractile amplitude before and during ischemia, as well as during reperfusion (Fig. 1). However, injection of DNIC in this dose prevented reperfusion fibrillations and cardiac arrest (Fig. 1).

At 200 μ/kg DNIC markedly decreased the depression of contraction amplitude and prevented heavt arrest during reperfusion (Fig. 1).

Injection of DNIC in a dose of 300 mg/kg produced no protective effect. Moreover, the initial amplitude of contractions was slightly decreased in comparison with that in control. This decrease can be an initial manifestation of the NO toxic effect.

Cardiac protection effect of DNIC revealed in this study is characterized by a distinct time-dependence (Fig. 2). The maximum resistance of isolated heart was observed 12 h after injection of the NO donor.

Thus, DNIC provides time- and dose-dependent protective effect and enhances the resistance of isolated heart to rhythm and contractile disorders during reperfusion.

Comparison of DNIC dynamics in blood and heart (Table 1) and the time course of enhancement

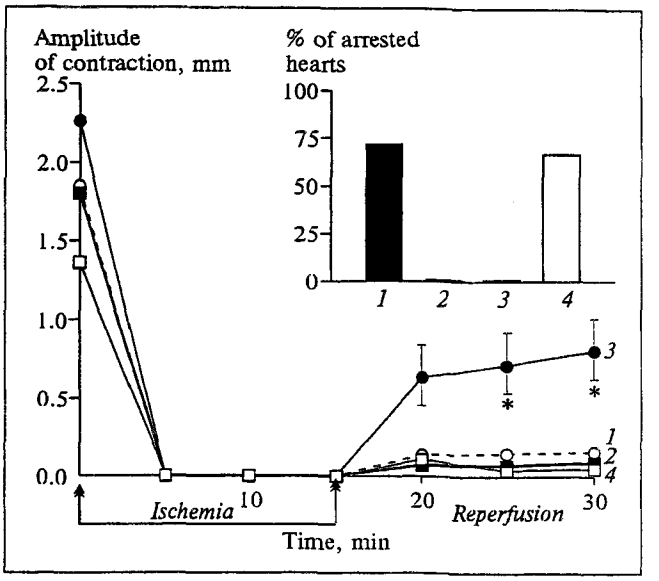

Fig.1. Dose-dependence of DNIC cardiac protective effect: *1*) control; doses of DNIC (mg/kg): 2) 100; 3) 200; *4*) 300. Here and in Fig. 2 and 3: *$p<0.05$, **$p<0.01$ in comparison with the control group.

of cardiac resistance to ischemic and reperfusion damage (Fig. 2) shows that protective effect of the NO donor occurs later than the transient increase of its content in heart and blood. Thus, the long-term DNIC protective effect may be due not to direct action of NO, but to some secondary NO-dependent mechanisms, which could be activated by increase in NO concentration.

At the next stage, we obtained the data indicating that one of these hypothetical mechanisms can be related to activation of the HSP70 synthesis. Figure 3 shows that the HSP transcription inhibitor quercetin completely prevented the development of the protective effect of NO donor.

Recently, anti-ischemic cardiac protective effect of NO donors was revealed in a number of studies [1]. This protective effect occurred either immediately after injection or during NO donor infusion at ischemic and reperfusion stages. First, this effect was supposed to result from a decrease in the endo-

TABLE 1. Dynamics of DNIC 1:20 (ng/kg tissue) in Blood and Heart after Injection of DNIC 1:2 (*M±m*, *n*=5)

Test object	Dose of DNIC 1:2, mg/kg								
	100			200			300		
	Postinjection time, h								
	1	12	24	1	12	24	1	12	24
Blood	277±6	0	0	587±67	0	0	1556±80	0	0
Heart	17±1	0	0	50±3	0	0	210±27	0	0

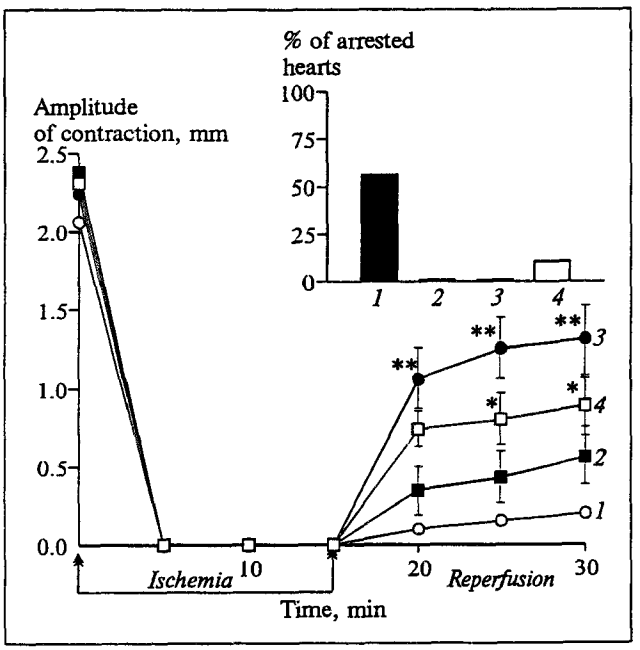

Fig. 2. Dose-dependence of DNIC cardiac protective effect on the time after its application. Time after DNIC injection: *1*) control; *2*) 1 h; *3*) 12 h; *4*) 24 h.

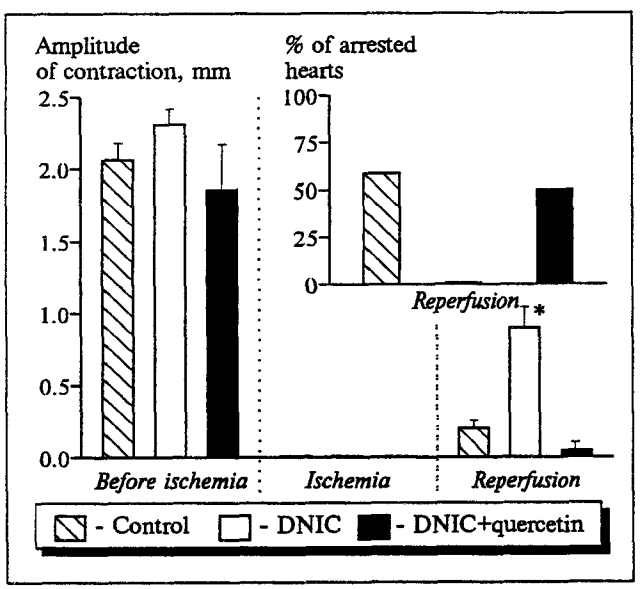

Fig. 3. The influence of quercetin on cardiac protective effect of DNIC.

thelial adhesion of neutrophils, a decrease in oxygen consumption during ischemia and reperfusion (mainly due to a decrease in heart rate), and an increase in coronary blood flow [1]. Since our experiments were performed on isolated heart, restriction of neutrophil adhesion cannot play any significant role in the protective effect of NO donor. In addition, heart rate did not change in isolated hearts of animals injected with NO the donor (data not shown).

Therefore, restriction in myocardial oxygen consumption also cannot cause protective effect. Finally, in our experiments protective effect took place 12-24 h after a single intravenous injection of NO donor, when the DNIC 1:20 content cannot be determined in heart and blood.

Thus, protective effect was provided not by NO itself, but by some secondary NO-activated mechanisms. The potency of NO donors to activate synthesis of HSP70 [6] and the effect of HSP70 transcription inhibitor on the NO protective effect (our finding) suggest that NO-dependent activation of HSP70 is the mechanism of cardiac protection against heart damage caused by ischemia and reperfusion.

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