Evaluation of Nitric Oxide Concentration in Isolated Organs by Nitric Oxide Microsensor

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Abstract: We applied a newly developed nitric oxide (NO) microsensor to the measurement of NO in isolated arteries and hearts. The change of NO concentration in canine femoral arterial wall by a change in perfusion flow rate and by NO synthase stimulation and inhibition using acetylcholine and N^G-nitro-*L*-arginine, respectively were successfully measured. The real-time changes of NO concentration in isolated rat hearts during changes of perfusion flow rate were also evaluated directly.

INTRODUCTION

Endothelium-derived nitric oxide (EDNO) plays a key role in regulation of circulation systems such as vascular tone and platelet adhesion, and thus the quantitative analysis of NO is of great importance for cardiovascular research. We have reported that using an NO microsensor we could measure a real-time change in NO concentration in the vascular media of isolated canine femoral arteries where NO mediates vascular tone during perfusion [1]. In this paper, we measured NO concentration in isolated arteries and hearts to evaluate the effects of perfusion flow rate (wall shear stress) and agonists on NO production.

METHOD

NO microsensors (100, 200 μmϕ; Inter Medical Corporation, Japan) were applied. The sensitivity and reliability of each NO sensor was evaluated using a solution of *S*-nitroso-*N*-acetylpenicillamine (SNAP). (i) The sensor was inserted into the vascular media of isolated canine femoral arteries using a micromanipulator. Each artery was perfused with a Krebs-Henseleit buffered solution, containing *L*-arginine (*L*-arg; 1 mM), NO synthase (NOS) agonist, acetylcholine (ACh; 1 mM), or NOS inhibitor, N^G-nitro-*L*-arginine (*L*-NA; 10 μM). The real-time change in NO current was monitored. (ii) An isolated rat heart was perfused under Langendorff mode with changing perfusion pressure (40-100 cmH_2O), and NO concentration in the right ventricle was measured. In addition, the change in NO concentration during transient flow-suspension and reperfusion was monitored. All these measurements were performed in an electromagnetically shielded system to exclude environmental electrical noise.

RESULTS AND DISCUSSION

NO concentration in the vascular media of femoral arteries was increased linearly with increasing perfusion flow rate (wall shear stress) (Figure 1). At a given perfusion flow rate, the measured NO concentration was increased by *L*-arg and ACh, and attenuated by *L*-NA. Thus, it was confirmed that the NO microsensor selectively measures NO.

NO concentration in the right ventricle was increased linearly with increasing perfusion pressure (40-100 cmH_2O), indicating flow-dependence of NO production in the heart. In the experiments of flow-suspension (1-10 min) and reperfusion, the measured NO concentration during reperfusion indicated the washout of NO accumulated in the myocardium during flow suspension and the increase in NO production, induced by reperfusion, i.e., an increase in wall shear stress.

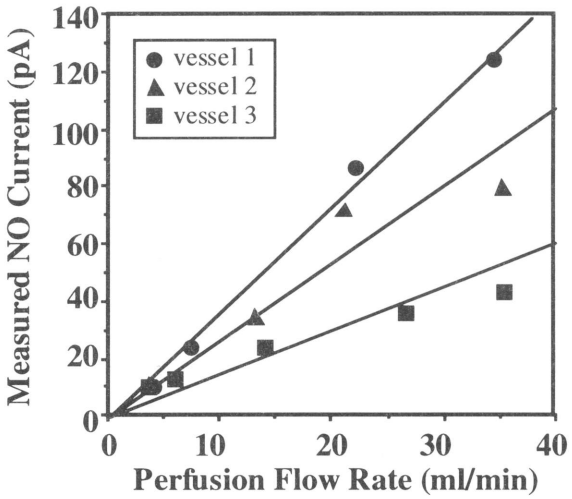

Figure 1. Effect of perfusion flow rate on NO release from isolated canine femoral arteries

CONCLUSIONS

The new NO sensor has satisfactory performance for NO measurement in the vascular media of isolated arteries and in the right ventricle of isolated hearts. The NO microsensor was thus found to have much potential for direct NO measurement in various cardiovascular systems.

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