Journal of Cardiovascular Pharmacology
17(Suppl. 3):S95–S99 © 1991 Raven Press, Ltd., New York

The Role of Nitric Oxide in the Control of Coronary Vascular Tone in Relation to Partial Oxygen Pressure, Perfusion Pressure, and Flow

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Summary: Isolated guinea pig hearts were used as a model to investigate the role of pO_2, coronary perfusion pressure (CPP), and flow on the release of nitric oxide (NO) into the coronary circulation. The in vitro half-life of NO strongly depends on the pO_2. Reduction of pO_2 from 700 to 50 mm Hg prolonged the NO half-life by 78%, from 3.6 to 6.4 s. Basal release of guansine-3',5'-cyclic monophosphate (cyclic GMP) taken as an index of endogenous NO concentration, remained unaltered in isolated hearts perfused at normoxic (100% O_2) and hypoxic conditions (30% O_2). Increase of CPP after infusion of 4 μM HbO_2 or 10 μM N^G-monomethyl-L-arginine (L-NMMA) (ΔCPP of 3 ± 0.1 and 2 ± 0.1%, respectively) was similiar during normoxic and hypoxic perfusion. Mannitol (25 mM) and dimethyl sulfoxide (10 mM), which are scavengers of hydroxyl radicals, significantly reduced CPP and increased basal cyclic GMP release. Bradykinin (100 nM) decreased CPP by more than 40% at each level studied. Increasing flow from 5 to 15 ml/min enhanced NO release from bradykinin-stimulated hearts from 61 ± 5 to 207 ± 13 pmol/min, determined by difference spectrophotometry. Maximal reduction of CPP by adenosine did not affect basal NO release. The presented data suggest that in vitro the half-life of NO strongly depends on pO_2. The NO half-life in situ may be influenced by the formation of hydroxyl radicals within the coronary circulation. Hypoxia-induced coronary vasodilation does not appear to be mediated by NO. NO release from bradykinin-stimulated hearts increases proportionally to enhanced flow rates. Basal release of NO occurs independently from changes in coronary perfusion pressure. **Key Words:** Endothelium-derived relaxing factor—Nitric oxide—Coronary circulation.

Endothelial cells contribute to the control of vascular tone and platelet function by the formation and release of various labile factors (for review, see refs. 1–4). Recently, an endothelium-derived relaxing factor (EDRF) was identified as nitric oxide (NO) (5–7) formed from the precursor amino acid L-arginine (8). NO plays an important role in the regulation of coronary vascular tone under basal and stimulated conditions (9,10). Prevention of NO formation from L-arginine results in an impairment of vessel conductance (11). Major determinants of coronary vascular conductance are pO_2, coronary pressure, and flow. The aim of the present study was to investigate whether or not the rate of endothelial NO release is modulated by these cardinal factors of vascular dynamics. Isolated, constant flow perfused guinea pig hearts were used as a model and NO release was quantified by a specific spectrophotometric assay (7,12).

MATERIALS AND METHODS

Isolated guinea pig hearts

Isolated hearts, from guinea pigs weighing 280–320 g, were electrically paced (285 beats/min) and perfused at constant flow according to the Langendorff technique with a medium containing (in mM) NaCl, 140; KCl, 4.0; $CaCl_2$, 1.84; $MgCl_2$, 1.03; NaH_2PO_4, 0.42; glucose, 5.0; pyruvate, 2.0; HEPES, 10.0; and indomethacin, 0.01 (pH 7.4, equilibrated with 100% O_2, at 37°C). Left ventricular pressure (LVP), dp/dt_{max}, and coronary perfusion pressure (CPP) were continuously documented on a Gould 2300 recorder (Oxnard, CA, U.S.A.); for further details, see refs. 9 and 10.

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Measurement of nitric oxide

Endothelial NO release was continuously quantified in the effluent perfusate by a specific difference-spectrophotometric assay, which previously has been used to measure NO release from organic nitrates, endothelial cells, and sydnonimines (7,12–14). The method is based on the rapid oxidation of oxyhemoglobin (HbO_2) to methemoglobin (MetHb) by NO. Measurement of the extinction difference (λ_1 of 401 nm, λ_2 of 411 nm) in a flow-through cell with a double-beam, double-wavelength spectrophotometer permitted the continuous assay of NO released into the coronary effluent perfusate (9,10).

Standards of aqueous NO solutions were prepared as previously described; the NO concentration of stock solutions was determined by means of high-performance liquid chromatography (HPLC) as nitrite ions (12). The half-life of NO in relation to the partial oxygen pressure (pO_2) of the perfusion medium was determined using a tubing system connected to the photometer, perfused with the medium described above at 2 ml/min, where sites of NO infusion were separated from the site of oxyhemoglobin infusion by different transit times (7,10).

Measurement of cyclic GMP

Guanosine-3',5'-cyclic monophosphate (cyclic GMP) release into the coronary effluent perfusate was determined with a commercially available radioimmunoassay (Amersham, Braunschweig, F.R.G.). The procedure of cyclic GMP separation from effluent samples and quantification by radioimmunoassay (RIA) has been described in detail previously (10).

Experimental protocols

To investigate the influence of pO_2 on the rate of NO formation and/or degradation, the following experimental series were performed: (a) The in vitro half-life of NO was measured at different pO_2 in the perfusion medium. (b) Cyclic GMP release, taken as an index for endogenous NO formation and release, was measured in the coronary effluent from normoxic and hypoxic perfused hearts under control conditions and in the presence of HbO_2 (4 μM) and N^G-monomethyl-L-arginine (L-NMMA); 100 μM). (c) Changes in CPP and release of cyclic GMP upon infusion of mannitol (25 mM) and dimethyl sulfoxide (DMSO, 10 mM) as scavengers of oxygen-derived hydroxyl radicals (·OH) were measured.

To evaluate the influence of changes in perfusion pressure and flow on the release of NO, isolated hearts were perfused (a) at flow rates of 5, 10, and 15 ml/min during bradykinin-stimulated conditions and (b) at constant flow (10 ml/min) while the CPP was reduced stepwise by increasing concentrations of adenosine. Bradykinin was chosen as a stimulus, because it has been shown to elicit maximal NO release from isolated guinea pig hearts, which persists upon repeated stimulation (15,16).

All compounds applied were of analytical grade and were tested for interference with the spectrophotometric assay for NO. Values are given as mean ± SEM.

RESULTS

In aqueous solution equilibrated with room air, NO is converted to nitrite (13). As shown in Fig. 1, the in vitro half-life of NO at 37°C was a function of the pO_2 in the perfusion medium (Fig. 1). The

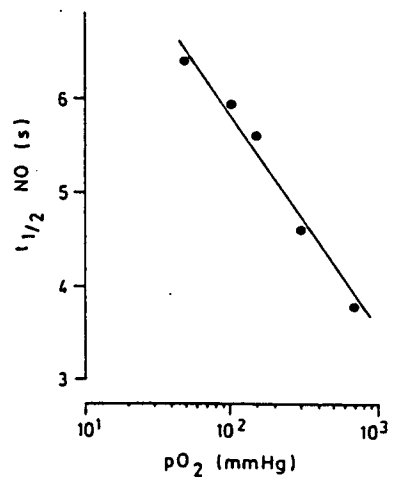

FIG. 1. Relationship between the in vitro half-life of nitric oxide (NO) and partial oxygen pressure (pO_2). The half-life of NO was determined spectrophotometrically in a tubing system perfused with a medium at different pO_2 (for further details, see the Methods section).

decay of NO to nitrite followed pseudo-first-order kinetics. Reduction of pO_2 from 700 to 50 mm Hg prolonged the NO half-life by 78%, from 3.6 to 6.4 s (Fig. 1). It thus seemed tempting to assume that the prolongation of the NO half-life at lower pO_2 levels is a determinant of the hypoxic vasodilation in vivo. To test this possibility, cyclic GMP was quantified in the coronary effluent perfusate of isolated hearts perfused under normoxic and hypoxic conditions (Fig. 2). Despite the pronounced hypoxia-induced reduction in CPP, the basal release of cyclic GMP remained constant. After switching back to normoxic perfusion, bradykinin, which elicited a coronary vasodilation similiar to that found with hypoxia, increased severalfold the basal cyclic GMP release, indicating that the soluble guanylate cyclase was not stimulated by hypoxia although it remained in a status to be stimulated.

The coronary vasoconstriction induced by 4 μM HbO_2 and 10 μM L-NMMA under normoxic conditions (ΔCPP of 3 ± 0.1 and 2 ± 0.1%, respectively, n = 4 each) was not significantly different from that during hypoxic perfusion. In parallel, the observed inhibition of basal cyclic GMP release was similiar during normoxic and hypoxic perfusion.

Besides molecular oxygen (O_2) and superoxide anions ($\cdot O_2^-$), hydroxyl radicals (·OH) may be involved in the breakdown of NO to NO_2^- (10). Mannitol and DMSO, which are scavengers of ·OH (17), were therefore infused into isolated guinea pig hearts and changes in CPP and cyclic GMP were recorded. Mannitol (25 mM) induced a pronounced coronary vasodilation (ΔCPP of −11 ± 3 mm Hg, =21%, n = 5), which was paralleled by a significant increase in basal cyclic GMP release (190 ± 11 fmol/min). Similiar results were obtained with DMSO,

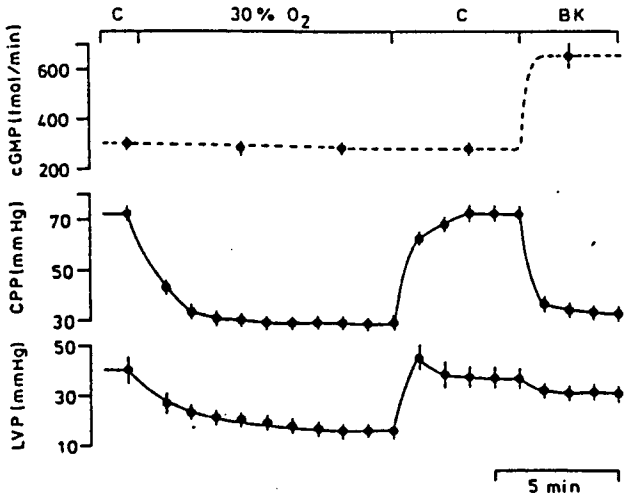

FIG. 2. Influence of hypoxic perfusion on basal cyclic GMP release. Isolated guinea pig hearts were perfused under normoxic (100% O_2) and hypoxic (30% O_2) conditions at 10 ml/min. Coronary perfusion pressure (CPP), left ventricular pressure (LVP), and the release of cGMP, as a marker of basal NO formation, were determined in parallel (n = 5). After hypoxic perfusion, hearts were stimulated with 100 nM bradykinin (BK) to demonstrate the responsiveness of guanylate cyclase.

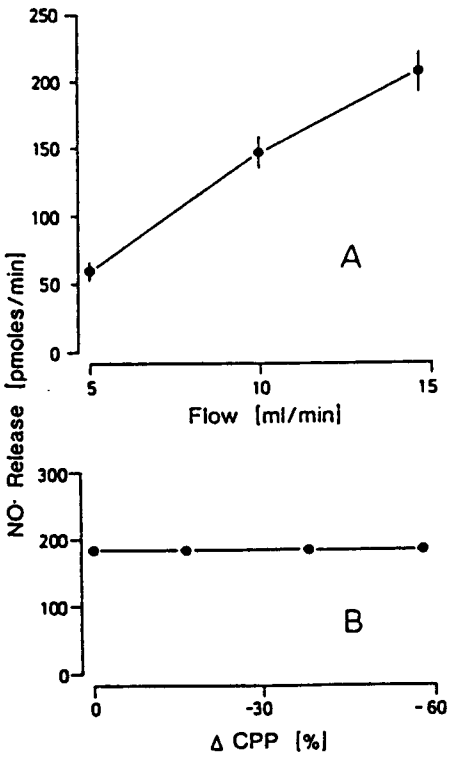

FIG. 3. Flow- and pressure-dependent release of nitric oxide (NO). (A) Isolated guinea pig hearts perfused with 5, 10, and 15 ml/min were stimulated with bradykinin (100 nM) and in parallel the bradykinin-induced enhancement of basal NO release into the coronary effluent perfusate was measured (n = 4). (B) Coronary perfusion pressure (CPP) of isolated hearts perfused at 10 ml/min was reduced stepwise by gradually increasing the concentration of infused adenosine (10^{-8}–10^{-5} M) whereas basal NO release was measured by difference spectrophotometry (n = 3). Changes in CPP are given as percent of control.

indicating that modulation of the ·OH concentration may affect the half-life of NO and by this the NO concentration within the coronary circulation.

To evaluate the role of coronary flow on the release of NO, bradykinin-stimulated isolated hearts were perfused at constant flows of 5, 10, and 15 ml/min and the NO release was quantified in the coronary effluent perfusate. Bradykinin, at a maximally effective concentration of 10^{-7} M, significantly decreased the CPP by more than 40% at all flow rates and increased NO release into the coronary effluent progressively with augmented flows from 61 to 207 pmol of NO/min (n = 4, Fig. 3A).

To separate flow- from pressure-dependent effects, isolated guinea pig hearts were perfused at a constant flow of 10 ml/min, while the CPP was progressively decreased by infusion of adenosine (from 10^{-8} to 10^{-5} M). Maximal adenosine-induced coronary vasodilation decreased the CPP approximately by 60% (EC_{50} = 0.2 μM) without affecting the LVP and dp/dt_{max}. Despite the substantial adenosine-induced decrease in CPP, the basal rate of NO formation remained constant (186 ± 30 pmol/min, n = 4, Fig. 3B).

DISCUSSION

The major findings reported here are as follows. (a) The in vitro half-life of NO strongly depends on the pO_2. (b) The NO half-life in situ may be influenced by the formation of hydroxyl radicals. (c) Hypoxia-induced coronary vasodilation does not appear to be mediated by NO. (d) NO is released into the coronary circulation in a flow-dependent manner. (e) Basal release of NO occurs independently from changes in coronary perfusion pressure.

Under in vitro conditions, the rate of NO degradation strongly depends on the pO_2 of the perfusion medium (Fig. 1). This in vitro half-life of NO is several times longer than that previously determined under in situ conditions (<130 ms) (10). Besides molecular oxygen and superoxide anions (18), hydroxyl radicals (10) were proposed to be involved in the rapid breakdown of NO. The reaction between ·OH and ·NO may form NO_2^-, the major product of NO degradation within the coronary circulation (10). In accordance with this view are the results obtained with mannitol and DMSO. Infusion of both compounds induced a coronary vasodilation, which was paralleled by a rise in the release of cyclic GMP. This effect may have been caused by its OH-scavenging properties (17), which may have prolonged the NO half-life and thus enhanced

the effective NO concentration. These findings support the interpretation that the effective NO concentration is likely to be regulated not only by its rate of formation but also by its rapid oxygen- and ·OH-dependent degradation.

As shown in Fig. 1, the half-life of NO is almost twofold prolonged by decreasing pO_2. This tempted us to study the effects of hypoxia on the formation of cyclic GMP during hypoxic perfusion. Although a pronounced coronary vasodilation was observed upon a change from normoxic to hypoxic perfusion, basal release of cyclic GMP, taken as a marker for basal NO release (10), remained unaltered (Fig. 2). This finding indicates that NO itself presumably is not involved in the hypoxia-induced coronary vasodilation and extends previously reported results, and that the endothelium of conduit arteries from canine and rabbit does not modulate hypoxia-induced vasodilation via the enhancement of cyclic GMP content in the vascular smooth muscle cells (19).

The lack of cyclic GMP rise during hypoxia can certainly not be explained by an impaired function of guanylate cyclase since infusion of bradykinin enhanced the cyclic GMP release strongly (Fig. 2). There remains a theoretical possibility that hypoxia itself may impair the activity of endothelial NO synthase. However, the results obtained with L-NMMA and HbO_2 furthermore support the interpretation that the hypoxic vasodilation occurs independently of endothelial NO synthesis. Both compounds prevent the relaxation of coronary resistance vessels induced by the basal release of NO via inhibition of synthesis or inactivation, respectively, resulting in a similiar degree of coronary constriction under normoxic and hypoxic conditions.

In conduit arteries (20,21) and in resistance-size vessels (22), endothelium-dependent and flow-induced dilation under basal and stimulated conditions has been described. For the coronary circulation, Inoue et al. have reported that flow-induced dilation in canine epicardial arteries following reactive hyperemia was markedly attenuated after endothelial denudation of the vessel (23). NO is a significant modulator of coronary vascular tone under both basal and stimulated conditions (10). In our experiments, stimulation of isolated guinea pig hearts with bradykinin resulted in an augmented release of NO (Fig. 3A) and a concomitant coronary vasodilation at each flow level studied. A threefold increase in flow elicited a proportional enhancement of bradykinin-induced NO release, providing that the NO concentration in the coronary effluent remained almost constant (Fig. 3A).

As a consequence of the increased flow, the NO concentration in the effluent perfusate remained almost constant although the total amount of released NO increased. This was taken as an indicator that the NO concentration at the luminal site of the endothelium was kept nearly constant. The NO concentration in the immediate vicinity of the surface of the endothelial cells may be even higher than those determined in the effluent, representing an average NO concentration released into the entire vessel lumen. Therefore, the antithrombogenic activity of endothelial cells with respect to NO, which prevents local platelet adhesion and aggregation (24), will be maintained regardless of the magnitude of blood flow. Disturbances of this protective mechanism may become relevant under pathophysiological conditions with endothelial dysfunctions such as atherosclerotic lesions of large and small coronary arteries.

Whether or not the NO concentration at the abluminal site of the endothelial layer following changes in flow is influenced to the same extent remains speculative as selective measurement of NO at the abluminal or interstitial space of the endothelium appears to be impossible.

Endothelial cells have been reported to modulate pressure-induced changes in arterial vessel diameter (25,26). According to this view, increases in perfusion pressure from 40 to 160 mm Hg induce a vasoconstriction that might be related to the release of a labile, endothelium-derived contracting factor. Inhibition of tonically released EDRF by infusion of oxyhemoglobin, however, had no effect on the vessel tone (26). The present study extends these functional studies, showing that the rate of basal NO release from isolated guinea pig hearts was not altered by changes in CPP induced by graded intracoronary infusion of adenosine (Fig. 3B). Thus, endothelial cells probably do not contribute to pressure-induced changes in vessel diameter by an altered formation and/or release of NO.

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