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Role of Nitric Oxide (NO) in the Regulation of Coronary Circulation

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Summary

Nitric oxide plays an important role in the control of basal coronary tone and mediation of reactive hyperaemic flow response following short-term coronary occlusion. The results presented in this report indicate that NO is involved in the modulation of coronary autoregulation in isolated rat hearts. Isolated rat hearts exhibit autoregulation of coronary flow (CF) between 50 and 80 cm H_2O of coronary perfusion pressure (CPP). Within this autoregulatory range NO release (measured as nitrite) varies from 1.7±0.3 to 2.2±0.7 nmol/min/g wt. Below the autoregulatory range it decreases slightly, while above this there is more than a twofold increase. Changes of NO release are accompanied by directly proportional changes of cGMP release. The release of hypoxanthine + xanthine shows a reciprocal relationship to CF values. The inhibition of NO synthesis showed a reciprocal relationship with CF values. Inhibition of NO synthesis by L-NAME (30 μmol/l) significantly reduces CF over the entire range of CPP changes (20–120 cm H_2O), but much less at lower than at higher pressure values. Therefore, the autoregulatory range is significantly widened to CPP of 40–100 cm H_2O. Theophylline (30 μmol/l) reduces CF by 15–25 % throughout the entire range of CPP changes. Hence, the CPP-CF curve is shifted downwards without significant changes of the autoregulatory range. Theophylline-induced reduction of NO release is CPP-dependent: as greater as CPP lower. When L-NAME is coadministered with theophylline, CF is additionally reduced while widened autoregulatory range is shifted to the right.

Key words

Nitric oxide – Rat heart – Coronary autoregulation – L-NAME – Theophylline

Introduction

The endothelium plays an important role in the control of vascular tone (Rees *et al.* 1989). Endothelial, nitric oxide-dependent control of coronary blood flow has attracted particular attention, although it seems that it is much more important in the kidney (Sonntag *et al.* 1992). Authentic NO, applied into the coronary circulation, decreases vascular resistance in a dose-dependent manner and enhances coronary release of cGMP (Kelm and Schrader 1990a). Kostić and Schrader (1992) have shown that NO is an important regulator of basal coronary flow as well as of reactive hyperaemic flow in isolated guinea-pig hearts. Later it was shown that NO is responsible for reactive hyperaemia (RH) which is evoked by short-term (1–10 s) coronary occlusion, whereas concurrent effects of NO and adenosine were required to maintain RH that followed longer (20–60 s) periods of the interruption of coronary inflow (Gryglewski *et al.* 1995). Furthermore, it has been shown that when NO synthesis is inhibited, adenosine is formed at an accelerated rate, which may compensate for the loss of NO-mediated vasorelaxation (Kostić and Schrader 1992). Inhibition of NO synthesis is also accompanied by inhibition of histamine release, which also regulates basal and RH flow in coronary circulation (Rosic *et al.* 1993).

Besides regulating RH, the vascular endothelium also modulates flow autoregulation (Rosenblum *et al.* 1987, Kuo *et al.* 1990). Coronary autoregulation is the intrinsic ability of the heart to maintain its nutritive blood supply relatively constant, despite the rather wide range of coronary perfusion pressure changes (Dole 1987). It reflects the interaction of myogenic tone with the opposing action of one or more endogenous vasodilators (Marcus 1983). Adenosine and histamine, as such vasodilators, do not appear to play any role in setting coronary tone within the autoregulatory range (Hanley *et al.* 1986,

Kostic and Jakovljevic 1996). However, recent data have shown that inhibition of NO production greatly alters the capacity of autoregulation in isolated buffer-perfused organs (Griffith and Edwards 1990, Ueeda *et al.* 1992).

The role of NO in the control of vascular tone during reactive hyperaemia (RH) has been proven in animals as well as in humans (Kostič and Schrader 1992, Tagawa *et al.* 1994, Gryglewski *et al.* 1995, Kostič and Jakovljevič 1996). Therefore, in the present article we present the results of investigations which were aimed to examine whether coronary autoregulation in isolated rat hearts is modulated by NO.

Materials and Methods

Hearts, isolated from Wistar albino rats of 200–300 g body mass, were perfused according to the Langendorff technique. The perfusion medium consisted of (in mmol/l) NaCl 118, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$ 2.5, $MgSO_4$ 1.7, KH_2PO_4 1.2, glucose 11, pyruvate 2. It was equilibrated with 95 % O_2+5% CO_2, warmed to 37 °C and delivered at an initial pressure of 60 cm H_2O. All hearts were electrically paced (4 V, 320 bpm). Constant left ventricular draining through the dissected mitral valve was performed.

After the isolated heart perfusion had been set up, 30 min was allowed for stabilization of the preparation. During this period, the hearts were challenged by short-term occlusions (5–15 s). After perfusion for 20–25 min, adenosine was injected (bolus of 60 μl, 5 mol/l adenosine at flow of 10 ml/min) in order to elicit maximum coronary flow. Hearts were discarded if flow did not increase by 100 % over the control. At the end of equilibration period, the coronary perfusion pressure was lowered to 50, 40, 30 and 20 cm H_2O, then increased gradually in the reverse order to 60 cm H_2O and further to 70, 80, 90, 100 and 110 or 120 cm H_2O. When flow was considered as stable at each value of perfusion pressure, samples of the coronary effluent were collected. At the end of this series of pressure changes (basic protocol), hearts were perfused with L-NAME (30 μmol/l), as an inhibitor of NO synthase (Emery 1995), in the experiments designed to estimate the role of NO in CA. In the second series of experiments the basic protocol was followed by perfusion with 30 μmol/l (final concentration) theophylline which, in this concentration, blocks the coronary vascular action of adenosine and ATP equally well (Borst and Schrader 1991). In the third series of experiments, perfusion with theophylline was performed from the beginning of the experiment and, after the first sequence of perfusion pressure changes protocol was repeated in the presence of theophylline (30 μmol/l) plus L-NAME (30 μmol/l).

Samples of the coronary effluent were collected after the stabilization of flow at each perfusion pressure value. NO was assessed as nitrite and quantified by the spectrophotometric method using the Griess reagent (Green *et al.* 1982). Lactate was determined enzymatically (Gutmann and Wahlefeld 1974). Samples of effluent perfusate were processed for analysis of metabolites released from rat hearts, as previously described (Kelm and Schrader 1990a,b). cGMP was determined with a commercially available radioimmunoassay (Amersham, Braunschweig, FRG). Hypoxanthine and xanthine were determined enzymatically (Jensen and Jorgensen 1989).

Statistical significance of differences was estimated using paired Student's t-test.

Results

The results of this study show that isolated rat hearts exhibit autoregulation of CF between 50 and 80 cm H_2O of CPP (Fig. 1), which is slightly different from the autoregulatory range in isolated guinea-pig hearts (Ueeda *et al.* 1992, Kostic and Petronijevic 1995). Within the autoregulatory range, NO release (Fig. 1) varied from 1.7±0.3 nmol/min/g (at 50 cm H_2O) to 2.2±0.7 nmol/min/g (at 80 cm H_2O). Below this autoregulatory range it decreased slightly to 0.8±0.1 nmol/min/g at 20 cm H_2O. However, above the autoregulatory range NO release increased more than twice as compared to values from the autoregulatory range. As shown in Fig. 1, the increase of NO release was accompanied with a 3-fold increase of cGMP release, which was in rat hearts 1663±324 fmol/min/g (n=4) on the average at a pressure of 70 cm H_2O. This value is several times higher than that reported for guinea-pig hearts (Kelm and Schrader 1990b, Kostic and Schrader 1992, Gryglewski *et al.* 1995). In contrast, the release of hypoxanthine and xanthine, degradative products of adenosine metabolism, showed a reciprocal relationship with CF as well as with the release of NO and cGMP. It declined from 53±19 nmol/min/g (at 30 cm H_2O) to 24±5 and 20±5 nmol/min/g at 70 and 120 cm H_2O, respectively. These values are almost one order higher than those reported for guinea-pig hearts (Schrader *et al.* 1977, Gryglewski *et al.* 1995).

The administration of 30 μmol/l L-NAME, but not D-NAME, significantly reduced CF over the entire range of perfusion pressure changes and widened coronary autoregulation to the range 40–100 cm H_2O (Fig. 2A). CF was particularly reduced above the autoregulatory range, as it was the case with NO release (Fig. 2B). Since NO reduces myocardial oxygen consumption (Node *et al.* 1995), we measured lactate release under basal conditions as well as in the presence of L-NAME (Fig. 2C). The administration of L-NAME abolished changes of lactate release and maintained it almost constant and independent of perfusion pressure.

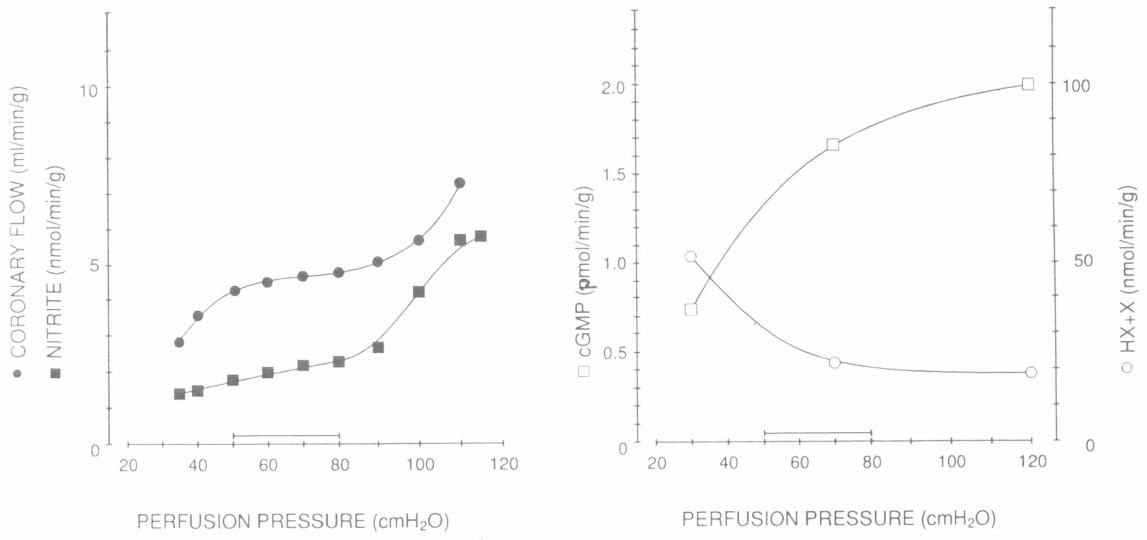

Fig. 1
Coronary autoregulation in the rat heart: Changes of coronary flow (CF) (full circles), nitrite (full squares), cGMP (open squares) and hypoxanthine + xanthine (HX+X) release (open circles), in relation to perfusion pressure. Horizontal bar denotes the autoregulatory range. Each point represents the means of four (cGMP, HX+X) and eleven (CF, nitrite) paired experiments.

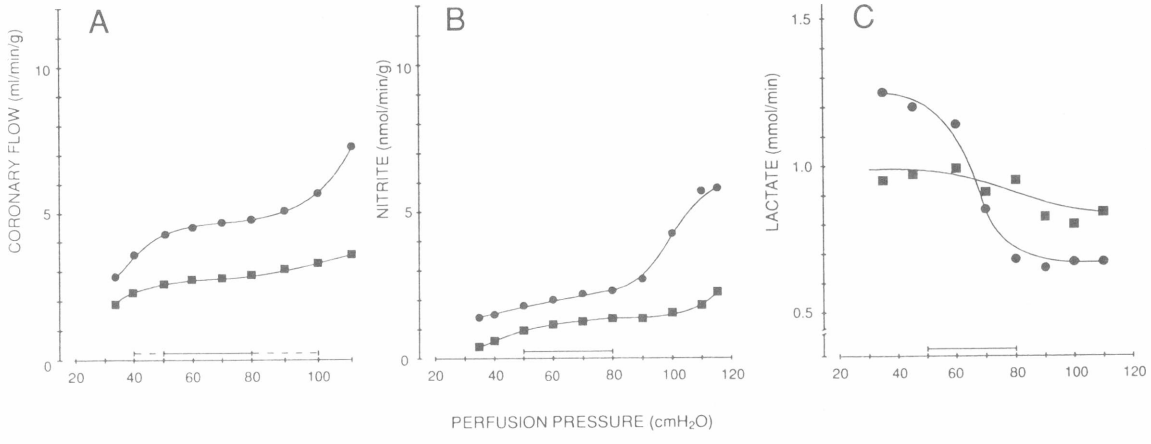

Fig. 2
Influence of L-NAME (full squares) on the coronary flow (A), nitrite (B) and lactate (C) release in relation to perfusion pressure. Full circles denote control values. Each point represents the means of eleven (A, B) and four (C) experiments.

Perfusion of the isolated rat heart with 30 μmol/l theophylline reduced CF by 15–25 % through the entire range of pressure changes (Fig. 3). Therefore, the CPP-CF curve was shifted downwards but the autoregulatory range was not significantly altered. These changes of CF were associated with marked impairment of NO release (Fig. 3). At CPP of 20 cm H_2O, the release of NO in the presence of theophylline amounted to 0.3±0.1 nmol/min/g which is 60 % less than in control hearts. At CPP of 30 and 70 cm H_2O, theophylline lowered NO release by about 45 %. At higher CPP, theophylline-induced inhibition of NO release gradually declined: at 120 cm H_2O it was about 30 %. When 30 μmol/l L-NAME was coadministered with theophylline, CF was additionally reduced, but much more at pressure values above 70 cm H_2O. Hence the autoregulatory range in these hearts was shifted to the right – between CPP values

of 60 and 120 cm H_2O. In the presence of theophylline plus L-NAME, the release of NO was reduced by 70–80 % although somewhat more at lower than at higher CPP values. The observed changes of cGMP release (not shown) were in accordance with the changes of released NO.

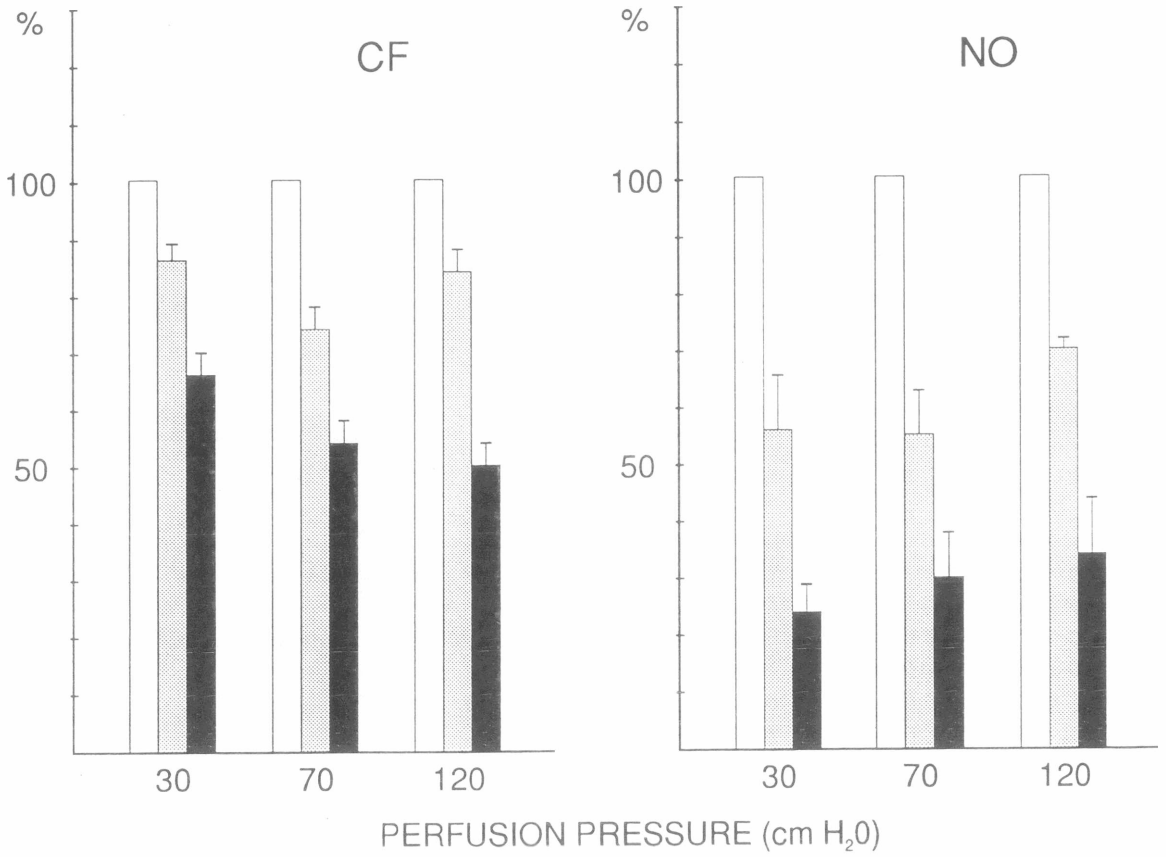

Fig. 3
Effects of 30 μmol/l theophylline (dotted columns) and 30 μmol/l theophylline + 30 μmol/l L-NAME (full columns) on coronary flow (CF) and nitrite (NO) release at different perfusion pressures. Control values (open columns) are represented as 100 %.

Discussion

Nitric oxide (NO) is an important endogenous regulator of coronary vascular and metabolic functions in the heart (Moncada *et al.* 1991, Kostić 1993). It is produced enzymatically from the semi-essential amino acid L-arginine (Palmer *et al.* 1988), the uptake of which by the heart occurs in two phases (Kostić *et al.* 1995). NO was thought to be formed only by the endothelial cells of coronary large vessels (Kelm and Schrader 1990a, Rees *et al.* 1990), but recent findings suggest that the cardiac myocytes are also able to synthesize NO (Schulz *et al.* 1992). It dilates coronary smooth muscles, inhibits the adhesion and aggregation of platelets, inhibits the activation of leukocytes and reduces myocardial oxygen consumption (Kelm and Schrader 1990b, Moncada *et al.* 1991, Kostić and Schrader 1991, Node *et al.* 1995).

It is well known that quantities of NO formed by the unstressed heart are sufficient to control basal coronary vascular tone (Kelm and Schrader 1990b, Kostic and Schrader 1992). Furthermore, NO is involved in mediating the reactive hyperaemic flow response in the isolated guinea-pig and rat hearts (Kostić and Schrader 1992, Gryglewski *et al.* 1995, Kostić and Jakovljević 1996). The results presented in this report clearly show the involvement of NO in modulation of coronary autoregulation in isolated rat hearts. In this respect, our work confirms findings of Ueeda *et al.* (1992). This work demonstrates changes of NO release for the first time although the changes were below and above the autoregulatory range, respectively (Fig. 1). The release of NO slightly increases throughout the autoregulatory range. However, above the autoregulatory range. the release of NO increases considerably, paralleling the increase of CF. Stimulation of NO release at high CPP and CF values is probably induced by increased shear stress and stretch (Ohno *et al.* 1990, Kelm *et al.* 1991, Ueeda *et al.* 1992). The release of NO at higher CPP values

appears to be particularly sensitive to the inhibitory action of L-NAME (Fig. 2B). Therefore, the autoregulatory range is widened mainly at the expense of the right part of the CPP-CF curve (Fig. 2A).

The release of cGMP into the effluent perfusate of isolated hearts reflects the formation of NO by coronary endothelial cells (Kelm and Schrader 1990a). According to our results (Fig. 1), the release of cGMP does not run in parallel to the release of NO over the entire series of performed pressure changes. What could be the reason for such a pattern of cGMP release? Here it may be assumed that other agents, released from the heart perfused at lower CPP, may stimulate the release of cGMP. Adenosine, the release of which is the higher the lower is CPP (Schrader *et al.* 1977), may be one of such agents since it is involved in the endothelium-dependent increase of cGMP release (Kurtz 1987, Moritoki *et al.* 1990). This is supported by our finding that theophylline, which does not influence coronary autoregulation in the rat heart, markedly suppresses NO (Fig. 3) and cGMP release (not shown) at lower CPP values. These results also indicate that adenosine and ATP released by the heart exert a vasodilatory action through the stimulated release of NO (Schrader *et al.* 1992, Minamino *et al.* 1995).

Ueeda *et al.* (1992) have shown that N^G-nitro-L-arginine (NNLA) significantly increases oxygen extraction, but especially at higher CPP values. These data are consistent with our results which showed that CPP has no influence on lactate release in the presence of L-NAME (Fig. 2C). The increased lactate release at lower CPP values, similarly as that after adenosine and histamine (Schrader *et al.* 1977, Kostić and Petronijević 1995, Kostić and Jakovljević 1996), is in accordance with the hypothesis that these metabolites are important for the regulation of coronary tone below the autoregulatory range. Therefore, their production, closely linked with cardiac metabolism, cannot explain the loss of the capacity to autoregulate at CPP values above the autoregulatory range. However, the increased release of NO above the autoregulatory range indicates that metabolites released from the heart may be a direct rather than an inverse function of CPP (Ueeda *et al.* 1992).

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