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Nitric Oxide Modulates Sympathetic Neurotransmission at the Prejunctional Level

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ABSTRACT In spite of accumulating evidence for a modulation of sympathetic neurotransmission by endogenously produced nitric oxide (NO), it remains unclear in which parts of the vascular system and at what level this interaction takes place. The aim of the present study was to investigate the distribution of endothelial and neuronal NO synthase (NOS) along the vascular tree of the heart at the light and electron microscopic level using NADPH-diaphorase (NADPH-d) staining as a marker for NOS. In addition, the functional effects of exogenous NO on coronary vascular resistance and cardiac adrenergic nerves was studied using the isolated perfused rat heart as a model. The intraaxonal catecholamine content of adrenergic nerve fibers was visualised and morphometrically assessed by applying glyoxylic acid-induced histofluorescence. The expression of endothelial NOS in the heart was found to depend on the diameter of the blood vessel. Arteries >100 μm always showed intense staining, whereas staining in smaller arteries and veins was considerably weaker. Smooth-muscle free vessels were essentially devoid of NADPH-d activity. In atrial and ventricular myocardium, neuronal NOS localised in autonomic nerve fibers along the entire vascular tree. Ultrastructurally, NADPH-d staining revealed adjacent localisation of NOS-positive and -negative axons, suggesting an interaxonal modulation of adjacent autonomic nerve fibers by NO. In isolated perfused rat hearts, the intracoronary application of 10^{-8} M NO produced a marked decrease of coronary perfusion pressure, which was accompanied by a distinct increase in intraaxonal catecholamine levels of intramural adrenergic nerve fibers. These results suggest that the entire vascular system from arteries to veins is under the influence of NO and implies that two independently operating NO-driven processes are involved in the modulation of blood vessel tone: the well-known pathway of endothelium-derived NO acting directly on smooth muscle, and a second indirect pathway that inhibits noradrenaline release from perivascular nerve endings by endothelially or neuronally produced NO. The uneven distribution of endothelial NOS furthermore suggests that the latter mechanism predominates when the size of the blood vessel decreases.

INTRODUCTION

The tone of a blood vessel is intimately controlled by two counterregulatory forces, namely, the tonic release of endothelium-derived nitric oxide (NO) and the release of noradrenaline from perivascular sympathetic nerves. The endogenous formation of NO has been demonstrated to be an important determinant of coronary vascular tone (Amezcua et al., 1989; Kelm and Schrader, 1990) and of coronary autoregulation (Ueeda et al., 1992). The NO-forming enzyme, NO synthase (NOS), resides not only in the vascular endothelium and in neurons of the central nervous system but is also present in discrete neuronal structures outside the brain, suggesting a role for NO as modulator of neurotransmitter release (Snyder and Bredt, 1991). Recently, the occurrence of NOS has been demonstrated in perivascular nerves innervating coronary vessels of the rat (Klimaschewski et al., 1992; Schmidt et al., 1992) and the guinea pig heart (Kummer et al., 1992; Tanaka et al., 1993), supporting the assumption that NO is involved in the neuronal control of the heart.

In vascular tissue noradrenaline was shown to induce endothelium-dependent relaxation via stimulation of endothelial α_2-adrenoceptors (Angus et al., 1986) and by this to counteract the vasoconstrictor action of noradrenaline (Cocks and Angus, 1983; Vanhoutte and Miller, 1989). Furthermore, it has been demonstrated that basal and bradykinin-stimulated EDRF release as well as the application of compounds that increase the level of cGMP reduce the efflux of noradrenaline from endothelium-denuded tissue (Cohen and Weisbrod, 1988; Greenberg et al., 1990, 1991). Increasing evidence suggests that the tonic release of

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NO may influence vascular tone not only by its direct vasodilator action on smooth muscle but also by an indirect action via modulation of sympathetic neurotransmission.

The first aim of the present study was to specifically investigate at the light and electron microscopic level the distribution of endothelial and neuronal NOS (Förstermann et al., 1991) along the vascular tree of the heart using NADPH diaphorase (NADPH-d) activity as a marker for NOS (Hope et al., 1991). The second aim was to study the functional effects of exogenously applied NO on coronary vascular resistance and cardiac adrenergic nerves using the isolated perfused rat heart as a model. The intraaxonal catecholamine content of cardiac sympathetic nerves was visualised by applying glyoxylic acid-induced histofluorescence.

MATERIALS AND METHODS

NADPH-Diaphorase Staining

Male Wistar rats weighing 400–500 g were anesthetised with pentobarbital. After opening the chest, hearts were perfused in situ with 500 ml of ice-cold phosphate buffered saline (PBS, pH 7.4) followed by 1,000 ml of a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) via a cannula inserted directly into the left ventricle. Hearts were excised, postfixed with 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C overnight, and then placed in PBS containing 18% sucrose. Free-floating 40–50 μm vibratome sections were incubated in 100 ml 0.1 M Tris/HCl buffer pH 8.0 containing 50 mg reduced nicotinamide adenine dinucleotide phosphate (NADPH), 100 mg nitroblue tetrazolium (NBT), 125 mg monosodium maleate, and 0.8% Triton X-100 at 37°C for 1 hour, then washed briefly in PBS. For the respective controls, NADPH was omitted from the incubation medium. For light microscopy, some sections were counterstained with cresyl violet and embedded in araldite for production of semithin sections of 3–4 μm. For ultrastructural examination, the slices were postfixed with 2% osmium tetroxide in 0.1 M PBS for 20 minutes at 4°C. This procedure gave rise to the formation of poorly soluble osmium-coordinated complexes with the formazan generated from NBT by the NADPH-d activity (Horobin, 1982), appearing as black spots at the ultrastructural level. Following a thorough wash in 0.1 M phosphate buffer for 30 minutes, the slices were dehydrated conventionally in a graded ethanol series and finally infiltrated and embedded in araldite. Ultrathin sections (30–60 nm) were obtained with a diamond knife on a Reichert ultramicrotome, placed on copper crids, and examined with a Zeiss EM 902A electron microscope.

Isolated Perfused Heart

Isolated heart perfusions were carried out on hearts from male Wistar rats weighing 190–230 g. Rats were anaesthetized with carbon dioxide (CO_2) and sacrificed by cervical dislocation. After opening the chest the heart was briefly rinsed with ice-cold saline, quickly excised after cannulation of the aorta, and retrogradely perfused according to the Langendorff technique (Langendorff, 1895). Hearts were perfused at a constant flow of 9 ml/min with a modified Krebs Henseleit buffer (composition [mM]: NaCl 126.8, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, glucose 5.0, $NaHCO_3$ 24.0, sodium lactate 1.0), prewarmed to 37°C, and equilibrated with 95% O_2/5% CO_2. Hearts were allowed to beat spontaneously and left ventricular pressure (LVP), coronary perfusion pressure (CPP), heart rate (HR), and maximum rate of rise of ventricular pressure (dp/dt) were continuously documented on a Watanabe linear recorder. Hearts were accepted for further study only when basal CPP was >85 mmHg. Pharmacological effects of NO were compared between age- and weight-matched groups of five animals each against the respective controls receiving saline only. After an intial equilibration period of 20 minutes, hearts were either perfused with saline or with an NO-containing solution at a final concentration of 10^{-8} M NO for 3 and 20 minutes, respectively. Isotonic aqueous solutions of NO were prepared daily and stored in gas-tight syringes as described previously (Feelisch, 1991). These NO-containing solutions were applied to the heart as a continuous infusion at a rate corresponding to less than 1% of coronary flow using a high precision infusion pump, whereas the outlet of the stainless steel tubing was placed directly in front of the coronary ostium.

Fluorescence Histochemistry of Catecholamines

After a 3 or 20 minute application of NO or saline, respectively, the right ventricles of isolated perfused hearts were rapidly cut off, freeze-clamped, and stored in liquid nitrogen for later histochemical examination of intraaxonal catecholamine stores. At a temperature of −30°C, the frozen myocardial tissue was cut into 16 μm cryostat serial sections. Adrenergic nerve fibres of the right ventricle were made visible by means of glyoxylic acid-induced fluorescence of intraneuronal catecholamines using the method of De la Torre (1980). Quantitative assessment of individual tissue sections was performed by high resolution microfluorimetry. The system was comprised of a Leitz orthoplan microscope equipped for fluorescence with epi-illumination and 3 mm BG 12 and Leitz K 490 primary and secondary filters, respectively. Using a residual light amplifying caesicon camera (Kranz Pic 762), tissues under study were focused in the way that within the visible field of the individual preparation only sections with nerve fibres running in parallel were processed, whereas sections containing sympathetic plexuses were excluded from measurement. After invertion of the primary image by means of a computerized image analysing system (Artek 982, Fisher Scientific), fluorescing adrenergic nerves were easily detectable as dark areas against a bright background (see Fig. 7). To eliminate background fluorescence, points below a given threshold intensity were filled up in brightness with the help of a specially designed measuring mask so that only fluorescing nerve fibres remained visible. The proportion of fluorescing areas in relation to the total area processed (3 mm^2) was estimated from the means of 100 consecutive measurements per section and was expressed on a percentage basis. No correction for fading of the fluorescence image by photodecomposition was necessary as the whole processing was per-

formed in 10 seconds. By using this technique, not only the length and thickness of the adrenergic nerve fibres itself but also the dimensions of their axonal dilatations, the "varicosities," were recorded.

RESULTS

NADPH-Diaphorase Activity in the Rat Heart

Light microscopic tissue examination revealed strong NADPH-d activity in the vascular endothelium of the rat heart. Dark blue reaction products were evenly distributed throughout the endothelial cell with the exception of the nucleus. The distribution of NADPH-d activity in endothelial cells along the vascular bed depended critically on the diameter of the blood vessel. Intramyocardial arteries of >100 μm in diameter always showed strong NADPH-d positive staining in their endothelial cells (Fig. 1,1a), suggesting that the entire endothelial cell layer of larger myocardial arteries contains NO synthase. In contrast, smaller arterial vessels displayed only isolated NADPH-d positive plaques in their endothelial cell layer (Fig. 2), the frequency of which clearly decreased with decreasing vessel diameter. Endothelial cells of arterioles very rarely displayed NADPH-d positive staining and, if present at all, this activity always was very weak. The smooth muscle-free terminal capillaries and postcapillary venules essentially were free of formazan reaction products (Fig. 2a). In contrast, endothelial cells of venous blood vessels showed weak NADPH-d staining in most cases.

Ultrastructural examination of NADPH-d activity of arterial and venous endothelial cells confirmed the results obtained at the light microscopic level. Besides its endothelial localisation, considerable activity was also demonstrated at the ultrastructural level in intracardiac nerve fibres, especially in axonal varicosities (Fig. 4). Parts of the axon bundles in the atrial myocardium and surrounding intramural and extramural vessels displayed diffuse distribution of electron dense spots. As in endothelial cells, a specific localisation of formazan precipitates in axonal organelles could not be demonstrated. Axonal and endothelial cell mitochondria were clearly free of NBT reaction products. Despite the negative staining of capillary endothelium, perivascular fibres innervating the capillaries showed positive NADPH-d staining (Fig. 3). Some nerve bundles contained positively and negatively stained axons side by side (Fig. 4), demonstrating selective localisation of NO-synthase in nerve fibres of the heart.

Hemodynamics of Isolated Hearts

Under the conditions of this study, isolated perfused rat hearts revealed the following baseline hemodynamic parameters: CPP 105 ± 14 mmHg, HR 314 ± 25 beats/min, +dp/dt 3690 ± 545 mmHg × s^{-1} (n = 20). Mean data obtained after 20 minutes of equilibration were not significantly different between groups (not shown). Intracoronary infusion of aqueous solutions of authentic NO produced a concentration-dependent reduction in CPP with half-maximal effects at about 10^{-8} M (data not shown), confirming the potent dilator effect of NO on coronary resistance vessels. Application of 10^{-8} M NO for 3 and 20 minutes produced a decrease in CPP of 31.7 ± 5.1% and 32.5 ± 4.0%, respectively, as compared to the corresponding control, whereas heart rate and contractility were not significantly affected.

Catecholamine-Related Fluorescence of Myocardial Nerve Fibres

Adrenergic nerve fibres were investigated 200 μm subepicardial. Nerve fibres running in parallel with cardiac muscle fibres displayed bright fluorescing axon varicosities. Application of NO produced both an increase in the number of visualised fluorescing nerve fibres and in the size of axon varicosities (Figs. 6 and 7). Under basal conditions, the area of fluorescing catecholamine stores corresponded to 1.88 ± 0.05% and 1.50 ± 0.08% of the total area processed upon saline perfusion of the hearts for 3 and 20 minutes, respectively. Application to the heart of 10^{-8} M NO for 3 and 20 minutes increased catecholamine related fluorescence by 26.1% and 24.7%, respectively, as compared to the control (Fig. 8).

DISCUSSION

By using NADPH-d staining we demonstrate here selective endothelial and neuronal localisation of NOS in the rat heart. The applied fluorescence histochemical technique provided evidence, for the first time, for a modulation of the intraaxonal content of catecholamines in cardiac adrenergic nerve fibres by exogenously applied NO, thus suggesting a role for NO in the regulation of sympathetic neurotransmission. Two important findings emerged from this study: firstly, NOS is unevenly distributed in endothelial cells along the vascular tree with decreasing expression from larger to smaller vessels and an essential lack of expression in the terminal vessels. Secondly, neuronal NOS activity is present in all perivascular nerves, regardless of the size of the innervated vessel. These results imply the involvement of two independently operating NO-driven processes in the modulation of blood vessel tone: one pathway where endothelium-derived NO acts directly on the smooth muscle cell, and another indirect pathway in which endothelium-derived or neuronally produced NO acts via inhibition of noradrenaline release.

The expression of NOS in endothelial cells appeared to depend on the diameter of the blood vessel. The most intense staining occurred in arteries with a diameter of >100 μm, and a considerably weaker staining was seen in smaller arterial and venous vessels. This finding is in agreement with a recent report from another group demonstrating that smaller arterioles contain very little NOS (Bredt et al., 1990). Blood vessels of the rat heart lacking smooth muscle, such as capillaries and postcapillary sinuses, did not reveal any detectable NADPH-d activity. The demonstrated augmentation of intraaxonal catecholamine content by exogenously applied NO provides further support for a feedback regulation between endothelial NO formation and the peripheral sympathetic system, and explains the finding of an enhanced efflux of noradrenaline following endothelial denudation (Cohen and Weisbrod, 1988; Greenberg et al., 1989, 1990).

The noradrenaline content of sympathetic nerve fi-

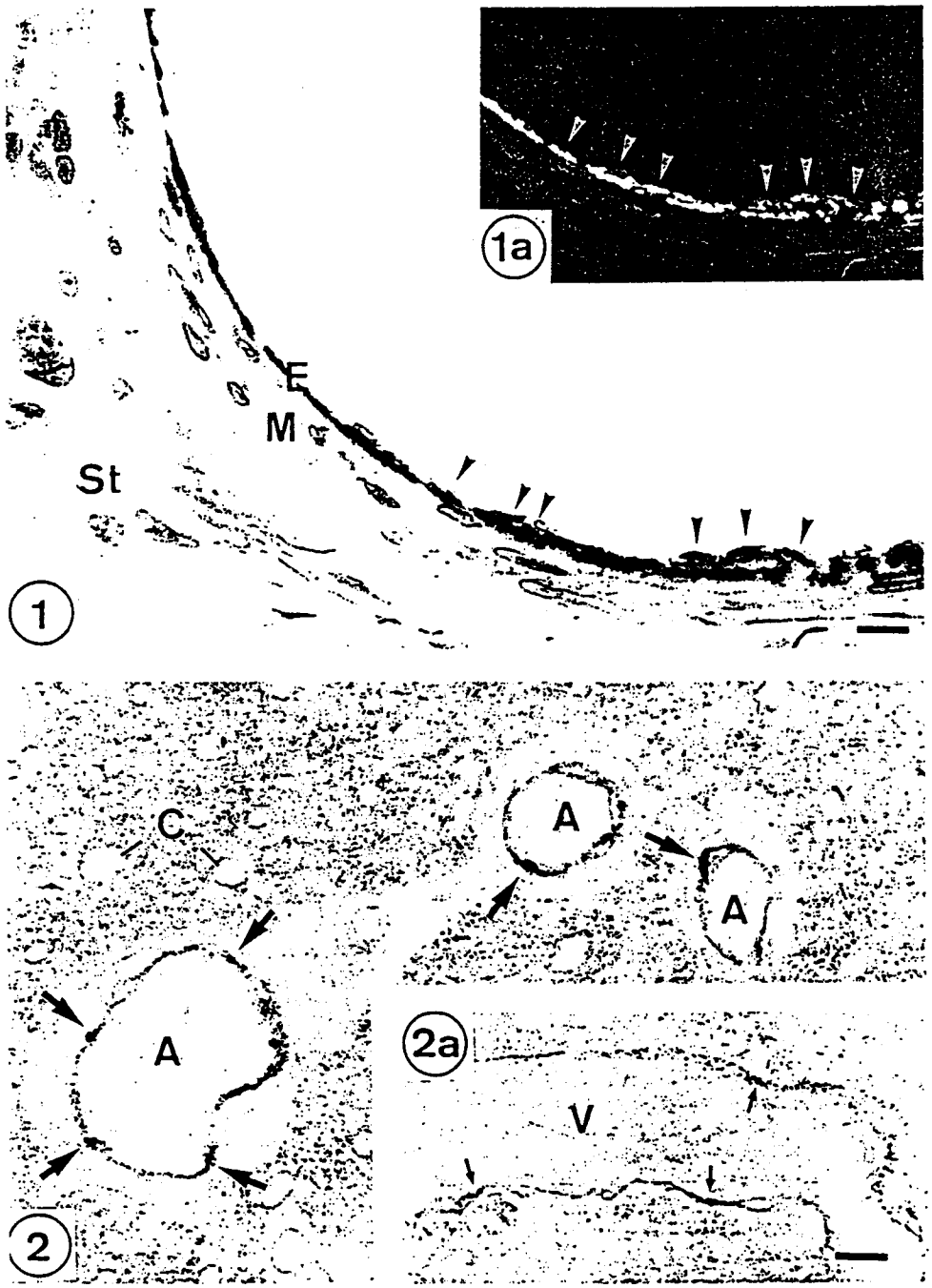
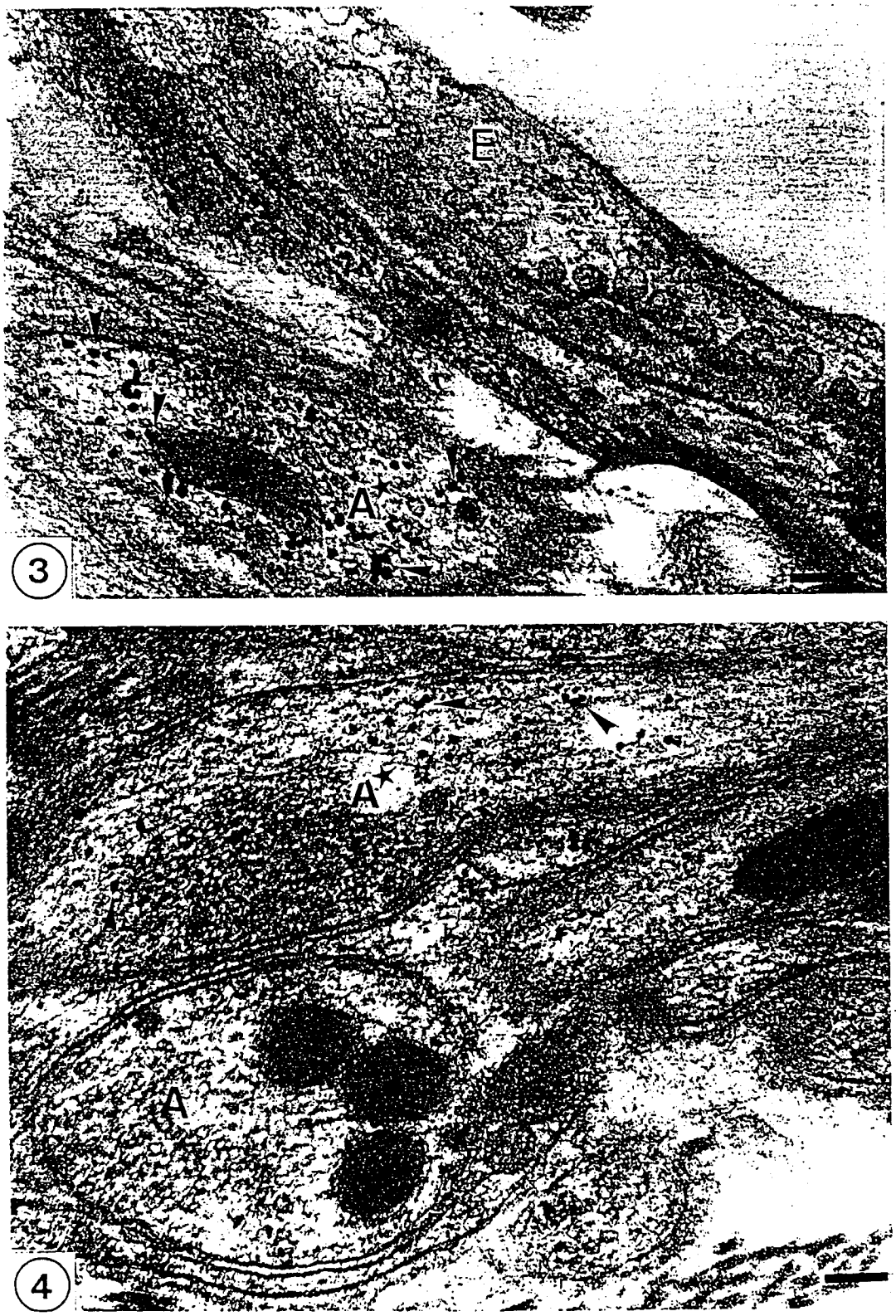


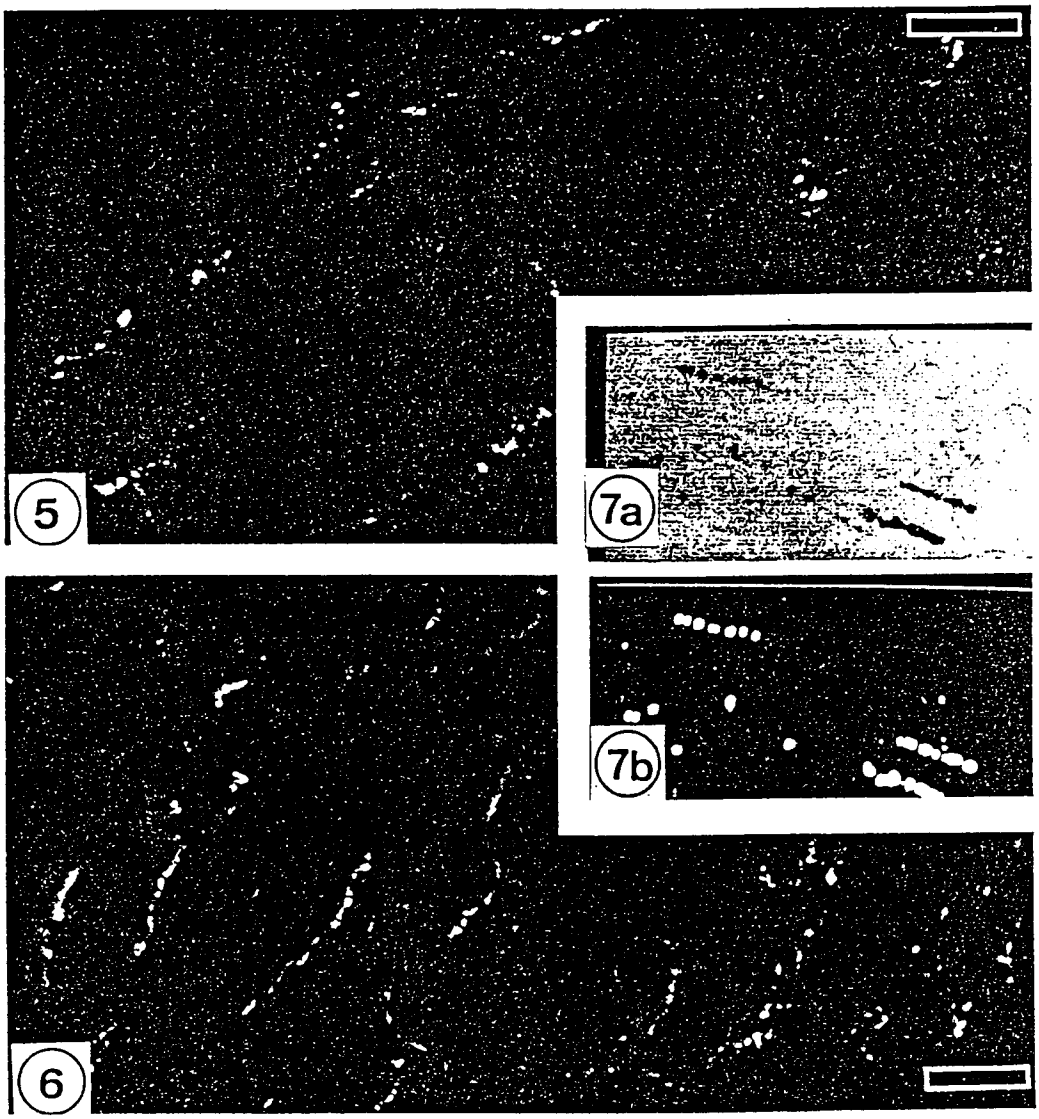
Fig. 1. Histochemical demonstration of NADPH-diaphorase in an artery of rat myocardium. Positive reaction is seen by darkening of endothelial cell bodies. All visible endothelial cells show positive NADPH-diaphorase reaction products. The individual positively stained endothelial cells are marked with an arrowhead (Fig. 1). E: endothelial cell, M: layer of smooth muscle cells, St: surrounding tissue. Image inversion shows a distinct white colouring of the arterial endothelium, which contrast against the darker surrounding tissue (Fig. 1a). Bar = 12 μm.

Fig. 2. Parts of the endothelial cells of rat intramyocardial arterioles (A) (Fig. 2) and venules (V) (Fig. 2a) show positive NADPH-diaphorase staining (arrows). Capillary endothelial cells (C) are devoid of NADPH-diaphorase reaction products (Fig. 3). Bar = 12 μm.

bers was visualised using glyoxylic acid-induced histofluorescence. Compared to the determination of tissue catecholamine content, this method allows differentiation between the intraaxonal and extraaxonal localisation of catecholamines in the myocardium and direct visualization of changes in intraneuronal storage sites that are otherwise difficult to examine. Using this technique a significant difference in intraaxonal



Figs. 3,4. Ultrastructural localization of formazan precipitates in rat myocardium following NADPH-diaphorase staining. Formazan precipitates appear as dark spots (arrowheads) in axons and axon varicosities. NADPH-diaphorase positive axons (A*) are found closely associated with capillary endothelial cells (E) (Fig. 3). Positively (A*) and negatively (A) stained axons appear adjacent to one another (Fig. 4). Bar = 250 nm.



Figs. 5,6. Glyoxylic acid induced catecholamine histofluorescence of intramural adrenergic nerve fibers in the right ventricle of the isolated perfused rat heart. After an equilibration period of 20 minutes, the hearts were perfused for 20 minutes with a perfusate containing nitric oxide at a concentration of 10^{-8} M or the vehicle alone. In control hearts, fluorescing nerve fibres course parallel to the axis of cardiomyocytes and form chainlike varicosities (Fig. 5). Following infusion of nitric oxide, a marked increase of catecholamine histofluorescence was observed, indicating an augmentation of intraaxonal catecholamines in myocardium (Fig. 6). Bar = 20 μm.

Fig. 7. Appearence of the intraaxonal catecholamine content in cardiac adrenergic nerve fibers after glyoxylic acid-induced catecholamine histofluorescence. After inversion on the monitor image, nerve fibers are displayed as black areas (a). Subsequent to elimination of points below a given threshold intensity (background fluorescence), the area of remaining points is determined with the aid of a specially designed measuring mask. Thus, only fluorescing nerve fibres are visible as white areas (b).

catecholamine content of nonstimulated isolated spontaneously beating hearts was observed between 3 and 20 minutes after equilibration, which is likely to reflect a continuous moderate release of catecholamines from the heart. The intracoronary application of a physiological concentration of NO (10^{-8} M) resulted in modulation of basal catecholamine levels in that it lead to an increase in glyoxylic acid-induced histofluorescence in the right ventricular myocardium. The rapid (within 3 minutes) augmentation of intraaxonal catecholamine levels persisted even after 20 minutes of perfusion with NO and suggests that in the heart, NO directly affects the steady state between release and reuptake of catecholamines and that this phenomenon is not prone to rapid tachyphylaxis.

Interestingly, the stimulation of sympathetic nerves in the heart leads to vasoconstriction of coronary arteries with larger diameter, whereas smaller arteries are known to respond with vasodilation (Moreland and Bohe, 1984). According to the results of the present

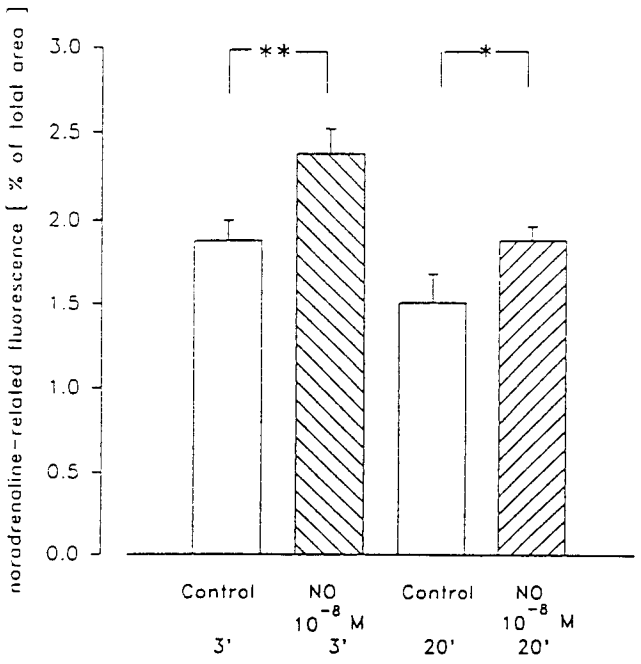


Fig. 8. Changes in catecholamine-related fluorescence of isolated perfused Langendorff hearts upon intracoronary infusion of authentic NO. Results are presented as the means ± SD of five individual experiments. Differences between group means were determined by calculation of one-way analysis of variance (ANOVA). Multiple comparisons were performed using adjusted t tests with P values corrected by the Bonferroni method (*$P < 0.05$; **$P < 0.01$).

study, the mechanism of inhibition of noradrenaline release by NO appears to operate predominantly in those parts of the coronary vascular bed that are constricted by noradrenaline. The weak expression of NOS in small arterioles, and the lack of expression in capillaries, fits the physiological evidence that endothelially supplied NO is more important in the regulation of large rather than small blood vessels.

At the ultrastructural level the NADPH-d staining technique demonstrated NOS activity in autonomic nerve fibres of the rat myocardium that innervate the blood vessels, particularly in axonal varicosities. These results are in accordance with findings by other groups (Bredt et al., 1990; Burnett et al., 1992; Klimaschewski et al., 1992; Kummer et al., 1992; Schmidt et al., 1992), who demonstrated blood vessel-associated NOS-containing nerve fibres in various tissues when applying the immunohistochemistry and NADPH-d techniques used in the present study. One important finding of our study is the universal perivascular localisation of NOS throughout all coronary vessels, despite the selective localisation of NOS in the endothelium of larger blood vessels. This may help to explain previously published data showing that part of the action of endogenous NO on the release of noradrenaline from vascular tissue still persists after complete removal of the endothelium (Greenberg et al., 1991).

We demonstrate side-by-side localisation of NADPH-d positive and negative nerve fibres in rat myocardium, which provides strong evidence for interneuronal communication between adjacent nerves via release of NO. Although no NADPH-d activity was found in capillary endothelial cells, the axonal varicosities of perivascular nerve fibres of the capillary wall, especially in rat myocardium, were, as a rule, NADPH-d positive. This implies that the entire vascular system from arteries to veins is under the influence of NO, and that regulation via inhibition of catecholamine release by neuronally produced NO becomes more and more important when the size of the blood vessel and the expression of endothelial NOS decreases. Our results furthermore suggest that NO modulation of catecholamine storage or release may have an important effect on capillary function. Several years ago, it became evident that the capillary system is also regulated by the activity of autonomic nerve fibres and that biogenic amines are involved in the modulation of capillary vessel diameter (Addicks et al., 1979; Weigel and Schwarzmann, 1981). Our results suggest that one such factor resonsible for the modulation of neurotransmitter release in the capillary bed of the heart may be NO. It further suggests that other factors, such as capillary permeability, may be under the control of NO released from perivascular nerve endings.

In spite of the association of adrenergic nerve fibers with the vascular system in the heart (Braunwald et al., 1964; Addicks, 1982), the implications of the present study are not necessarily limited to the regulation of coronary blood flow and vascular tone but may extend to other metabolic events in the heart. Recent evidence suggests that the endocardium of isolated perfused hearts is involved in the control of cardiac contractility (Ramaciotti et al., 1992; Shah et al., 1991). Because of the similarity between the effects seen in this study with those known to be produced by catecholamines, it appears conceivable that an NO-mediated modulation of noradrenaline release may account for this phenomenon. Until now, only very few data are available on the regulation of the neuronal synthesis of NO in the heart. Hence, a detailed analysis of the functional role of nitrergic nerve fibers and their interaction with the adrenergic nervous system remains to be established.

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REFERENCES

Addicks, K. (1982) Zur Innervation des Herzens. In: Autonome Innervation des Herzens. B. Brisse and F. Bender, eds. Steinkopff-Verlag, Darmstadt, pp. 1–13.

Addicks, K., Weigelt, H., Hauck, G., Lübbers, D.W., and Knoche, H. (1979) Light- and electronmicroscopic studies with regard to the role of intraendothelial structures under normal and inflammatory conditions. Bibl Anat., 17:21–35.

Amezcua, J.L., Palmer, R.M., de Sousa, B.M., and Moncada, S. (1989) Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. Br. J. Pharmacol., 97:1119–1124.

Angus, J.A., Cocks, T.M., and Satoh, K. (1986) Alpha2-adrenoreceptors and endothelium-dependent relaxation in canine large arteries. Br. J. Pharmacol., 88:767–777.

Braunwald, E.D., Harrison, D.C., and Chidsey, C.A. (1964) The heart as an endocrine organ. Am. J. Med., 36:1–4.

Bredt, D.S., Hwang, P.M., and Snyder, S.H. (1990) Localization of

nitric oxide synthase indicating a neural role for nitric oxide. Nature, 347:768–770.
Burnett, A.L., Lowenstein, C.J., Bredt, D.S., Chang, T.S.K., and Snyder, S.H. (1992) Nitric oxide: A physiologic mediator of penile erection. Science, 257:401–403.
Cocks, T.M., and Angus, J.A. (1983) Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature, 305:627–630.
Cohen, R.A., and Weisbrod, R.M. (1988) Endothelium inhibits norepinephrine release from adrenergic nerves of rabbit carotid artery. Am. J. Physiol., 254:H871–878.
De la Torre, J.C. (1980) An improved approach to histofluorescence using the SPG-method for tissue monoamines. J. Neurosci. Methods, 3:1–6.
Feelisch, M. (1991) The biochemical pathways of nitric oxide formation from nitrovasodilators: Appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. J. Card. Pharmacol., 17(Suppl):25–33.
Förstermann, U., Schmidt, H.H.H.W., Pollock, J.S., Sheng, H., Mitchell, J.A., Warner, T.D., Nakane, MM, and Murad, F. (1991) Isoforms of nitric oxide synthase. Characterization and purification from different cell types. Biochem. Pharmacol., 42:1849–57.
Greenberg, S., Diecke, F.P.J., Peevy, K., and Tanaka, T.P. (1989) The endothelium modulates adrenergic neurotransmission to canine pulmonary arteries and veins. Eur. J. Pharmacol., 162:67–80.
Greenberg, S.S., Diecke, F.P., Peery, K., and Tanaka, T.P. (1990) Release of norepinephrine from adrenergic nerve endings of blood vessels is modulated by endothelium-derived relaxing factor. Am. J. Hypertens., 3:211–218.
Greenberg, S.S., Peery, K., and Tanaka, T.P. (1991) Endothelium derived and intraneuronal nitric oxide-dependent inhibition of norepinephrine efflux from sympathetic nerves by bradykinin. Am. J. Hypertens., 4:464–467.
Hope, B.T., Michael, G.J., Knigge, K.M., and Vincent, S.R. (1991) Neuronal NADPH diaphorase is a nitric oxide synthase. Proc. Natl. Acad. Sci. USA, 88:2811–2814.
Horobin, R.W. (1982) Histochemistry: An Explanatory Outline of Histochemistry and Biophysical Staining. Gustav Fischer Verlag, Stuttgart, pp. 148–150.
Kelm, M., and Schrader, J. (1990) Control of coronary vascular tone by nitric oxide. Circ. Res., 66:1561–1575.
Klimaschewski, L., Kummer, W., Mayer, B., Couraud, J.Y., Preissler, U., Philippin, B., and Heym, C. (1992) Nitric oxide synthase in cardiac nerve fibers and neurons of rat and guinea pig heart. Circ. Res., 71:1533–1537.
Kummer, W., Fischer, A., Mundel, P., Mayer, B., Hoba, B., Philippin, B., and Preissler, U. (1992) Nitric oxide synthase in VIP-containing vasodilator nerve fibers in the guinea pig. NeuroReport, 3:653–655.
Langendorff, O. (1895) Untersuchungen am überlebenden Säugetierherzen. Pflügers Arch. Ges. Physiol., 61:291.
Moreland, R.S., and Bohe, D.F. (1984) Adrenergic control of coronary arteries. Fed. Proc., 43:2857–2861.
Ramaciotti, C., Sharkey, A., Mcclellan, G., and Winegrad, S. (1992) Endothelial cells regulate cardiac contractility. Proc. Natl. Acad. Sci. USA, 89:4033–4036.
Schmidt, H.H.W., Gagne, G.D., Nakane, M., Pollock, J.S., Miller, M.F., and Murad, F. (1992) Mapping of neural nitric oxide synthase in the rat suggests frequent co-localization with NADPH diaphorase but not with soluble guanylyl cyclase, and novel paraneural functions for nitrinergic signal transduction. J. Histochem. Cytochem., 40:1439–1456.
Shah, A.M., Lewis, M.J., and Henderson, A.H. (1991) Effects of 8-bromo-cyclic GMP on contraction and on inotropic response of ferret cardiac muscle. J. Mol. Cell Cardiol., 23:55–64.
Snyder, S.H., and Bredt, D.S. (1991) Nitric oxide as a neuronal messenger. Trends Pharmacol. Sci., 12:125–128.
Tanaka, K., Hassall, C.J.S., and Burnstock, G. (1993) Distribution of intracardiac neurones and nerve terminals that contain a marker for nitric oxide, NADPH-diaphorase, in the guinea-pig heart. Cell Tissue Res., 273:293–300.
Ueeda, M., Silvia, S.K., and Olsson, R.A. (1992) Nitric oxide modulates coronary autoregulation in the guinea pig. Circ. Res., 70: 1296–1303.
Vanhoutte, P.M., and Miller, V.M. (1989) Alpha 2-adrenoceptors and endothelium-derived relaxing factor. Am. J. Med., 87:1–5.
Weigel, H., and Schwarzmann, V. (1981) A new method for the simultaneous presentation of low and high magnification of microscopic specimens: Application to in vivo studies of mesenterial capillaries. Microsc. Acta, 85:161–173.