Endogenous and exogenous nitrates and their role in myocardial ischaemia

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- 1 Although nitrates have been prescribed in patients with angina pectoris for more than a century, their mechanism of action has only been understood recently.
- 2 The discovery of the endogenous nitrovasodilator nitric oxide, which is formed in endothelial cells by the enzyme nitric oxide synthase, has greatly expanded our knowledge. Nitric oxide, if released from endothelial cells can interact with vascular smooth muscle as well as circulating blood cells such as platelets. Nitric oxide activates soluble guanylate cyclase, which in turn leads to an intracellular increase in cyclic GMP. In vascular smooth muscle, this causes vasorelaxation, in platelets dysaggregation and prevention of platelet adhesion. This protective pathway both reduces the effects of vasoconstrictor substances, can produce profound vasodilation, if activated appropriately and acts as a regulator of platelet-vessel wall interaction. In addition, nitric oxide inhibits the production and action of endothelin, a 21 amino acid vasoconstrictor peptide formed by endothelial cells.
- 3 Exogenous nitrovasodilators also exert their action by releasing nitric oxide from the molecule. Their action is particularly pronounced in blood vessels with a low basal production of nitric oxide and is enhanced after removal of the endothelium. In coronary artery disease, the formation of endothelium-derived nitric oxide is reduced, its breakdown is increased, but only at later stages, is the action of endogenous and therapeutic nitrates depressed.
- 4 Hence, nitrates are an appropriate therapeutic tool in patients with coronary artery disease to substitute the effects of the impaired activity of the endothelial L-arginine/nitric oxide pathway.

Keywords EDRF endogenous nitrates exogenous nitrates myocardial ischaemia

Introduction

Nitrovasodilators have been used in patients with myocardial ischaemia ever since Thomas Lauder Brunton first used amyl nitrite to treat angina pectoris more than 100 years ago (Abrams, 1988; Brunton, 1887; Lüscher & Vanhoutte, 1990). The discovery that endothelium-derived relaxing factor (EDRF) represents an endogenous nitrate has greatly expanded our understanding on the use and mechanism of action of nitrovasodilators (Figure 1; Lüscher & Vanhoutte, 1990).

Endothelium-derived relaxing factor (EDRF): the endogenous nitrovasodilator

In 1980, Furchgott & Zawadzki reported that the relaxing properties of acetylcholine depend on the presence

of a functional endothelium (Figure 2). The mediator of endothelium-dependent relaxation (previously referred to as EDRF) has now been identified as nitric oxide (NO; Palmer *et al.*, 1987). Nitric oxide activates soluble guanylate cyclase in vascular smooth muscle (Rapoport *et al.*, 1983). Thus, the relaxation induced by endothelium-derived nitric oxide as well as nitrovasodilators such as nitroglycerin, sodium nitroprusside and 3-morpholino-sydnonimine (SIN-1; the active metabolite of molsindomine) is preceded by an increase in the content of cyclic GMP in vascular smooth muscle (Figure 1; Lüscher & Vanhoutte, 1990; Rapoport *et al.*, 1983). The degree of accumulation of the nucleotides correlates with the decreases in tension obtained. The pharmacological activity of the various nitrovasodilators in isolated vascular tissues correlates with the amount of nitric oxide that

Figure 1 Comparison of the effect of nitrovasodilators and endogenous nitric oxide (NO) which is formed from L-arginine in endothelial cells. Nitroglycerin and related compounds must be metabolised in order to be able to release the NO radical which in turn activates soluble guanylate cyclase (sGC). In order to form the nitrosothiol intermediate, sulph-hydryl groups are required which may be important in the development of nitrate tolerance. SIN-1, the active metabolite of molsidomine, has similar vascular and antiplatelet effects as nitric oxide, while other nitrates are much less effective in inhibiting platelet function. cGMP = cyclic GMP; GTP = guanosine triphosphate; L-NMMA = L-N^G-monomethyl arginine (from Lüscher & Vanhoutte (1990), with permission).

Figure 2 Effects of acetylcholine in the human internal mammary artery with endothelium (top panel) and in a preparation without endothelium (lower panel). In the ring with, but not in that without endothelium, acetylcholine causes full relaxation of the noradrenaline-induced (NA) contraction. The inhibitor of nitric oxide formation L-N^G-monomethyl arginine (L-NMMA) markedly reduces the response (data from Yang *et al.* (1991a), with permission of the American Heart Association).

Figure 3 Basal formation of endothelium-derived nitric oxide in intramyocardial coronary resistance arteries: the inhibitor of nitric oxide formation L-N^G-monomethyl arginine (L-NMMA) causes an increase in tension in rings with endothelium (top panel), but not in that without endothelium (lower panel). The endothelium-dependent contraction to L-NMMA can be reversed by L-, but not D-arginine (from Tschudi *et al.* (1991), with permission).

they release (Bredt & Snyder, 1990; Feelisch & Noack, 1987).

Nitric oxide is liberated from the amino acid L-arginine in endothelial cells via the activity of a specific cytosolic enzyme which is calcium-dependent and requires calmodulin and NADPH (Bredt & Snyder, 1990; Palmer *et al.*, 1988; Yang *et al.*, 1991a). Nitric oxide is liberated from the vascular endothelium under basal conditions (Figure 3; Lüscher & Vanhoutte, 1990; Tschudi *et al.*, 1991) and in response to a variety of stimuli such as shear stress (exerted by the circulating blood; Pohl *et al.*, 1986; Rubanyi *et al.*, 1986), circulating hormones (such as noradrenaline, vasopressin and bradykinin; Lüscher & Vanhoutte, 1990) and various autacoids (such as acetylcholine, histamine, substance P; Figure 2; Furchgott & Zawadzki, 1980; Lüscher & Vanhoutte, 1990; Lüscher *et al.*, 1988; Yang *et al.*, 1991a).

Interestingly, substances derived from aggregating platelets (such as adenosine diphosphate (ADP) and serotonin; Förstermann *et al.*, 1988; Yang *et al.*, 1991b) or from the coagulation cascade (such as thrombin) also release the endogenous nitrate NO and thereby are an important determinant of platelet-vessel wall interaction (Figure 4). Indeed, human aggregating platelets release enough ATP and ADP to evoke endothelium-dependent relaxations in arteries with an intact endothelium. In contrast, in the absence of endothelial cells, aggregating platelets cause marked contractions which are mediated by serotonin and thromboxane A₂ (Figure 4; Yang *et al.*, 1991b). Platelets contain the enzyme guanylate cyclase and form cyclic GMP after exposure to EDRF and nitric oxide. The increase in intracellular cyclic GMP is associated with a decreased platelet adhesion and aggregation (Radomski *et al.*, 1987a,b).

Thus, under physiological conditions, the endothelium protects the blood vessel wall against vasoconstrictor stimuli (Figure 5) exerted by circulating hormones and/or activated platelets (Förstermann *et al.*, 1988; Lüscher *et al.*, 1990a; Yang *et al.*, 1991a, b). For the latter property of the endothelium, the platelet inhibitory action of endothelium-derived nitric oxide is of great physiological importance.

Figure 4 Endothelium-derived nitric oxide and platelet-vessel wall interaction: platelets release substances such as adenosine triphosphate and -diphosphate (ATP, ADP), thromboxane A₂ (TXA₂) and serotonin (5-HT). In addition, at sites where platelet are activated thrombin (Thr) is formed. All these mediators can activate receptors on the endothelium and vascular smooth muscle (open circles). Thrombin and adenine nucleotides are potent activators of the release of endothelium-derived relaxing factor (EDRF) which is an inhibitor of platelet function (–) and potent vasodilator. Both effects are mediated by cyclic GMP (cGMP). In addition, the endothelium is a source of the vasoconstrictor peptide endothelin which is produced in response to thrombin and noradrenaline (NA) (from Lüscher *et al.* (1990a), by permission).

Endothelial effects of endogenous and exogenous nitrovasodilators

Endothelium-derived nitric oxide has pronounced effects on endothelial cells. Indeed, after activation of the L-arginine pathway, the intracellular cyclic GMP levels markedly increase (Schini *et al.*, 1989). Similar effects can be obtained with exogenous nitrovasodilators. It is unlikely that the increase in cyclic GMP in endothelial cells plays (as a negative feed-back mechanism) an important role in the regulation of the release of

Figure 5 Modulation of contractility by basally formed endothelium-derived nitric oxide in human arteries (mean $\pm$ s.e. mean results): in the internal mammary artery with endothelium, the inhibitor of the nitric oxide formation L-N^G-monomethyl arginine (L-NMMA) augments both the sensitivity and maximal response to noradrenaline, while the agent does not alter the responsiveness of preparations without endothelium (from Yang *et al.* (1991a), with permission of the American Heart Association).

Figure 6 Basal formation of endothelium-derived nitric oxide and responsiveness to nitrovasodilators (mean $\pm$ s.e. mean results): in porcine intramyocardial coronary arteries, the vasodilator effects of 8-morpholino sydnonimine (SIN-1; the active metabolite of molsidomine) are reduced in preparations with (left panel) as compared with those without (right panel) endothelium. In arteries with endothelium, the inhibitor of nitric oxide formation L-N^G-monomethyl arginine (L-NMMA) causes a similar augmentation in the sensitivity to the nitrovasodilator as endothelium removal suggesting that the basal formation of the endogenous nitrate reduces the effects of exogenous nitrovasodilators (from Tschudi *et al.* (1991), with permission).

endothelium-derived nitric oxide (Lüscher & Vanhoutte, 1990). However, nitric oxide and nitrovasodilators inhibit the thrombin-induced stimulation of endothelin production from intact vascular tissue via a cyclic GMP-dependent mechanism (Boulanger & Lüscher, 1990 and 1991). Indeed, superoxide dismutase (to inhibit the breakdown of endothelium-derived nitric oxide), 8-bromo-cyclic GMP and nitrates such as SIN-1 (the active metabolite of molsidomine) and nitroglycerin prevent the thrombin-induced release of endothelin from the intact porcine aorta. Thus, this regulatory mechanism of the production of endothelin provides a new mechanism of action of nitrates in the vasculature (Lüscher *et al.*, 1991).

Endothelium-dependent modulation of the effects of nitrovasodilators

The basal formation of endothelium-derived nitric oxide not only affects the reactivity of isolated blood vessels to vasoconstrictor substances (Figure 5), but also the effects of nitrovasodilators (Figure 6; Lüscher *et al.*, 1989, 1990a; Pohl & Busse, 1987; Shirasaki & Su, 1985; Tschudi *et al.*, 1991). Indeed, removal of the endothelium augments the relaxations induced by nitrovasodilators such as sodium nitroprusside, nitroglycerin, and SIN-1. The inhibitory effect of the endothelium can be prevented with the inhibitor of nitric oxide formation L-N^G-monomethyl arginine (L-NMMA) indicating that this related to basally released EDRF (Figure 6; Tschudi *et al.*, 1991). Bioassay experiments with cultured bovine aortic endothelial cells, confirmed the concept that endothelium-derived relaxing factor reduces the effects of nitrovasodilators on vascular smooth muscle (Pohl & Busse, 1987). Hence, different levels of basal release of endothelium-derived nitric oxide may explain why the endothelium inhibits the effects of nitrates in certain but not in all blood vessels (Lüscher *et al.*, 1989; Yang *et al.*, 1991a). Alternatively, the nitrovasodilators may stimulate

the endothelial cells to produce superoxide anions, breaking down the nitric oxide released by the compounds and thus reduces its effective concentration at the level of the vascular smooth muscle (Flavahan & Vanhoutte, 1989).

The reduced basal formation of endothelium-derived nitric oxide in veins as compared with arteries (Lüscher *et al.*, 1989; Yang *et al.*, 1991a), may also explain why veins are more sensitive to the effects of nitrovasodilators (particularly, if contracted with norepinephrine) as compared with arteries (Figure 7). In contrast, the venous vascular smooth muscle loses its sensitivity to the effects of nitrovasodilators, if contracted with endothelin (Lüscher *et al.*, 1990b). Indeed, under these conditions,

Figure 7 Effects (mean $\pm$ s.e. mean) of exogenous nitric oxide in the human internal mammary artery (IMA) and internal mammary vein (IMV). Note the enhanced sensitivity to nitric oxide in the vein as compared with the artery.

the responsiveness to the nitrovasodilator is blunted in veins contracted with endothelin-1 as compared with those contracted with norepinephrine or as arteries contracted with either vasoconstrictor.

Differences and similarities between exogenous and endogenous nitrates

Exogenous and endogenous nitrates differ in several aspects. First of all, endothelium-derived nitric oxide is not a circulating hormone, but rather a locally produced substance which primarily is regulated and acts in an autocrine fashion (Lüscher & Vanhoutte, 1990). Accordingly, local factors and particular functional properties of the endothelium in a given part of the circulation determine the regulatory role of endothelium-derived nitric oxide. In contrast, therapeutic nitrates are either given orally or intravenously and thus primarily act in a systemic fashion. Hence, systemic application of exogenous nitrates or stimulation of the production of endogenous nitrates does not necessarily lead to the same physiological effects.

Although the common property of all exogenous nitrates and the L-arginine pathway in endothelial cells is the liberation of nitric oxide (Feelisch & Noack, 1987) and in turn activation of soluble guanylate cyclase (Rapoport *et al.*, 1983), the biochemical pathways used to that end differ considerably. Hence, in particular nitrates such as nitroglycerin undergo a complex biotransformation requiring sulph-hydryl groups in order to release nitric oxide (Abrams, 1986; May *et al.*, 1987). This mechanism is prone to tachyphylaxis and plays an important role in the pathogenesis of nitrate tolerance. In contrast to nitroglycerin, SIN-1 and the endogenous nitrate formed from L-arginine do not develop tolerance (Berkenboom *et al.*, 1988; Kuhn & Förstermann, 1989; Kukovetz & Holzmann, 1983; Stewart *et al.*, 1987).

Some, but not all nitrates exert potent inhibitory effects on platelets. In particular, sodium nitroprusside and SIN-1 increase the content of cyclic GMP in platelets and inhibit aggregation *in vivo* and *in vitro* as well as the production of thromboxane A₂ by the cells (Basista *et al.*, 1985; Block *et al.*, 1982; Darius *et al.*, 1984; Gerzer *et al.*, 1989; Wirthumer-Hoche *et al.*, 1984; Yamakado

et al., 1982). The inhibition of the conversion of labelled arachidonic acid to thromboxane B₂ is much less pronounced with isosorbide dinitrate than with SIN-1, nitroglycerin or isosorbide-5-mononitrate (Wirthumer-Hoche *et al.*, 1984). These effects of certain nitrates may have important therapeutic implications for the prevention of acute ischaemic syndromes in patients with coronary artery disease (Fiedler, 1981; Just & Martorana, 1989).

Endogenous and exogenous nitrates in myocardial ischaemia

Myocardial ischaemia predominantly occurs in patients with coronary artery disease. Under these conditions, major epicardial coronary arteries undergo atherosclerotic changes. *Atherosclerosis* primarily is a disease involving the intimal layer of the blood vessel wall (Ross, 1986). Although the endothelium morphologically remains intact in atherosclerotic blood vessels (except in very late stages and at sites of plaque rupture), atherosclerosis is associated with pronounced functional alterations of the endothelium. Indeed, in atherosclerotic human coronary arteries, endothelium-dependent relaxations to acetylcholine, histamine, substance P and aggregating platelets are reduced (Figure 8; Bossaller *et al.*, 1987; Förstermann *et al.*, 1988). Although the sensitivity to nitroglycerin can be reduced particularly at sites of more advanced lesions, the maximal response is well-maintained. This suggests that a decreased endothelium-dependent relaxation occurring in atherosclerosis is primarily related to a decreased release of endothelium-derived nitric oxide (Shimokawa & Vanhoutte, 1989a).

Decreased endothelium-dependent relaxations also occur in *hyperlipidaemia* before angiographically detectable atherosclerotic changes develop (Shimokawa & Vanhoutte, 1989b). Indeed, oxidised low-density lipoproteins (which are present in early atherosclerotic plaques; Ylä-Herttuala *et al.*, 1989) specifically interfere with the endothelial L-arginine pathway and thereby reduce endothelium-dependent relaxations to aggregating platelets in large coronary arteries (Kugiyama *et al.*, 1990; Simon *et al.*, 1990; Tanner *et al.*, 1991).

Ischaemia and reperfusion also lead to pronounced

Figure 8 Endothelium-dependent relaxations and atherosclerosis (mean $\pm$ s.e. mean results): in coronary arteries obtained from patients with coronary artery disease, endothelium-dependent relaxations to acetylcholine, substance P and histamine are markedly reduced (closed symbols) as compared with those obtained from patients without atherosclerotic changes.

* = statistically significant difference between coronary arteries with and without atherosclerosis (data from Bossaller *et al.*, 1987).

Figure 9 Ischaemia and reperfusion and endothelium-dependent relaxations in porcine coronary arteries: while in control arteries, serotonin (in the presence of ketanserin to inhibit 5-HT₂-serotonergic receptors on vascular smooth muscle) evokes potent endothelium-dependent relaxations. In contrast, after ischaemia and reperfusion, endothelium-dependent relaxations to the monoamine are markedly reduced and the contractions are augmented. PGF_{2α} = prostaglandin F_{2α} (from Lüscher *et al.* (1990a), with permission).

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alterations in endothelial function. In the coronary circulation of the dog and pig, occlusion of a main coronary artery for 20 min or more followed by reperfusion for 1 h is associated with marked decreases in endothelium-dependent relaxations (Figure 9; Ku, 1982; Lüscher *et al.*, 1990a; Pearson *et al.*, 1990a; Van Benthuyssen *et al.*, 1987). As the response to exogenous nitrovasodilators is well-maintained, this again suggests that ischaemia and reperfusion are associated with a decreased formation or release of endothelium-derived nitric oxide. Of particular clinical interest is the fact that these changes appear to persist for up to 3 months (Pearson *et al.*, 1990b). Thus, prolonged ischaemia and reperfusion may alter coronary vasomotion and platelet-vessel wall interaction, a functional alteration which may contribute to the progression and the complications of coronary artery disease. Nitrates may be of particular importance under these conditions as their effects appear to be well-maintained.

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