Ulster says ‘NO’; explosion, resistance and tolerance
Nitric oxide and the actions of organic nitrates

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Those who have started to read this review expecting a discussion of the political situation in Northern Ireland will either be relieved or disappointed. The title refers to the discovery and actions of nitric oxide and the role played by investigators in Northern Ireland, its relationship to the explosive nitroglycerine and the concepts of nitrate resistance and tolerance in cardiovascular medicine.

In 1867 Thomas Brunton, a newly qualified house surgeon at the Edinburgh Royal Infirmary pioneered the clinical use of the sphygmomanometer by employing it to monitor the increase in blood pressure associated with episodes of angina pectoris. Brunton speculated that amyl nitrite, a drug which he had observed to lower blood pressure in animals, might be used to relieve angina pectoris. He obtained a sample of amyl nitrite and poured it on to a cloth for his patient to inhale. Within a minute the pain disappeared and the patient remained free of pain for several hours. Along with other nitrates and nitrites Brunton investigated the compound nitroglycerine which had become widely available following Alfred Nobel’s discovery of its property as an explosive. Later the following year, William Murrell, a registrar at the Westminster Hospital decided to resolve the conflicting reports in the literature as to whether nitroglycerine caused severe headache or not. The French chemist, Ascagne Sobrero, who had first synthesised the compound, reported in 1847 that he experienced an intense headache after tasting a small drop placed on his finger. Others had similar experiences including Arthur Field, a Brighton dentist who claimed in the Medical Times and Gazette that if placed on the tongue it could alleviate toothache and neuralgia. He contended that it would be a highly effective remedy for these conditions if it were not for the severe accompanying headache. In contrast, others reported no effects whatsoever after swallowing large amounts of nitroglycerine. Murrell suspected that the differences were largely due to variation in the susceptibility of different individuals, and decided to conduct an experiment in healthy volunteers. In the course of one of his outpatient clinics he licked the moist cork of the bottle containing nitroglycerine solution and within a few seconds began to experience a throbbing headache and a pounding in the chest. After five minutes he had recovered sufficiently to resume his clinic but the headache persisted for most of the afternoon. With further experimentation, Murrell determined three important pharmacological properties of nitroglycerine; the effect lasted longer than amyl nitrite, the headache was usually related to overdosage and larger doses were required if the drug was swallowed than if it was allowed to dissolve slowly in the mouth. Murrell treated his first patients with nitroglycerine in 1878 and the following year published his report in the Lancet. As a result, nitroglycerine was introduced into clinical practice for the treatment of angina pectoris. British doctors took steps to avoid any unnecessary alarm that might result if patients discovered that they were receiving the same explosive that was in dynamite and renamed the compound glyceryl trinitrate. No such niceties were adopted by their American colleagues, and in the United States the drug is still known as nitroglycerin. The high lipid solubility of glyceryl trinitrate facilitates good absorption across the buccal mucosa and the poorer response seen after oral absorption relates to first pass metabolism
by the liver. The headache is due to the well-described vasodilator effects of nitrates on the extra-cranial blood vessels.

Despite widespread use of organic nitrates for over a century, the method by which they dilated veins and arteries was unknown. A new chapter in cardiovascular physiology and pharmacology opened in the early nineteen eighties with the discovery of a hitherto unknown vasodilator substance produced by the vascular endothelium EDRF (endothelium derived relaxing factor). This compound is one of the most active endogenous vasodilators and is involved in the modulation of blood vessel tone. It took almost eight years of intense research to establish that this vasodilator compound was one of the smallest molecules known, NO (nitric oxide). Organic nitrates enter vascular smooth muscle cells where they are reduced by sulphhydryl groups to liberate nitric oxide. This in turn activates the enzyme soluble guanylate cyclase which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) resulting in reduced intracellular calcium concentrations and smooth muscle relaxation (Figure 1). Sodium nitroprusside is an inorganic nitrate which forms nitric oxide directly without the need for biotransformation. It is a useful tool for assessing vascular smooth muscle responsiveness to nitric oxide, and distinguishing between impaired dilator responses which are due to an abnormality in the vascular smooth muscle, and those due to an endothelial defect. Recent observations have suggested that resistance to the action of nitrates occurs in a significant percentage of the population probably due to deficiency of intracellular sulphhydryl donors, and this is more commonly seen in diabetes mellitus and heart failure. Continuous administration of organic nitrates can also lead to vascular tolerance through a similar mechanism. Repeated doses probably result in fewer S-nitrosothiols, leading to reduced activation of guanylate cyclase and a lower concentration of cyclic GMP with a resulting decrease in vascular relaxation.

THE PHYSIOLOGY AND PHARMACOLOGY OF NITRIC OXIDE

Endothelial cells secrete nitric oxide which relaxes vascular smooth muscle. Under physiological conditions NO is constantly released and ensures patency of the blood vessel. In the endothelial cells, NO is synthesised from the amino acid L-arginine by NO-synthase. This enzyme is continuously active ensuring the constant release of NO. Disturbances in endothelial cell function decrease the production of NO and thus reduce vasodilatation. In addition to NO, endothelial cells also release prostaglandins with vasodilator activity such as prostacyclin. This endoperoxide induces vasodilation and inhibits platelet aggregation. Damaged or excessively activated endothelial cells can also secrete vasoconstricting factors of which the recently discovered endothelin-1 is the best known. Although thromboxane, a powerful vasoconstrictor, is produced by activated platelets, it is also released by damaged endothelial cells (Figure 2).

Three nitric oxide syntheses have been described; an endothelial isoform (eNOS), a neuronal isoform (nNOS) in nitrergic nerves and a macrophage or inducible isoform (iNOS). The genes encoding the three isoforms of NO synthase have been located on human chromosome 7 (eNOS), 12 (nNOS) and 17 (iNOS). The two predominant isoforms of NOS are eNOS and nNOS. The inducible form of the enzyme is iNOS found in endothelial, smooth muscle and several
other cells. It is involved in host defence mechanisms and immunological reactions. The constitutive form of NO synthase is expressed in endothelial cells and is involved in the regulation of vascular tone, structure and haemostasis. A wide range of factors have been found to stimulate the constitutive form of NO synthase including mechanical factors (shear stress and pulsatile pressure), platelet products (bradykinin, serotonin and histamine) and neurohormonal mediators (angiotensin II, acetylcholine and noradrenaline). Arteries produce more nitric oxide than veins, although veins respond better to organic nitrodrilators. The biological half life is approximately thirty seconds and it is rapidly inactivated by haemoglobin, methylene blue and the superoxide anion. The development of competitive inhibitors of NO synthase, the L-arginine analogues N\textsuperscript{0} monomethyl-L-arginine (L-NMMA) and NO\textsuperscript{0} nitro-L-arginine methyl ester (L-NAME) has provided an invaluable tool for investigation of the role of NO in biological processes. In addition to movement of nitric oxide from the endothelium to vascular smooth muscle, nitric oxide is also released into the lumen of blood vessels. Here it acts synergistically with prostacyclin to inhibit platelet aggregation, smooth muscle growth and cell proliferation.\textsuperscript{18}

There is increasing evidence that nitric oxide has important physiological effects throughout the body and production is not confined to the vascular endothelium. Nitric oxide is present in lung epithelium and other pulmonary cells and has been suggested as the mediator of nerve dependent bronchodilatation.\textsuperscript{19} Reduced nitric oxide release may also be the mechanism underlying pulmonary vasoconstriction which occurs secondary to hypoxia\textsuperscript{20} and inhaled nitric oxide has been shown to reduce pulmonary vasoconstriction and the elevated pulmonary pressure occurring in chronic hypoxia. There is recent evidence that nitric oxide also has an important role in the central nervous system in protecting neurons from degeneration and mediating neuronal activity and nutritive blood flow. In high concentrations however, there is experimental evidence to suggest that nitric oxide may cause neuronal damage.\textsuperscript{21, 22} Other functions of nitric oxide include control of gastrointestinal sphincter tone and motility,\textsuperscript{23} the release of insulin in diabetic patients,\textsuperscript{24} penile erection,\textsuperscript{25} regulation of lymphocyte function\textsuperscript{26} and producing vasodilation and tissue leakage in the inflammatory response.\textsuperscript{27}

**ENDOTHELIAL DYSFUNCTION AS A MARKER FOR VASCULAR DISEASE**

The discovery of Furchgott and Zawadzki in 1980 of the obligatory role of the endothelium in producing vascular smooth muscle relaxation in response to acetylcholine\textsuperscript{3} has provided a valuable method for assessing endothelial function. This method has been widely used to assess endothelial function in vivo in animals and humans. Most in vitro studies of endothelium-dependent relaxation involve mounting an isolated artery in an organ bath. The length of the artery is fixed and changes in isometric tension recorded. The artery is preconstricted with noradrenaline or phenylephrine and the relaxation in response to an endothelium-dependent vasodilator such as acetylcholine or serotonin recorded. In order to confirm that impaired vasodilatation is due to endothelial dysfunction, relaxation in response to an endothelium-independent vasodilator, usually sodium nitroprusside or glyceryl trinitrate is also determined. This helps to exclude non-specific impairment of smooth muscle relaxation. The method has been used to demonstrate impaired endothelial function in animals with diabetes,\textsuperscript{28} hypercholesterolaemia,\textsuperscript{29} hypertension\textsuperscript{30} and vessels with evidence of atherosclerosis.\textsuperscript{31} A small number of studies investigating endothelial function in smaller blood vessels have been carried out in diabetic animals examining pial,\textsuperscript{32} retinal\textsuperscript{33} or mesenteric\textsuperscript{34} arterioles. Saenz de Tejada et al 1989 demonstrated that isolated penile corpora cavernosum tissue from diabetic and control subjects taken at the time of penile prosthesis implantation had impaired responses to acetylcholine.\textsuperscript{35} The technique of venous occlusion plethysmography has been widely used to assess endothelial function in human subjects. This method involves the measurement of forearm blood flow in response to endothelium-dependent and independent vasodilators infused locally into the brachial artery.\textsuperscript{7} It is the method which we in the Department of Therapeutics and Pharmacology have used most frequently to assess endothelial function in a variety of diseased states. Alternatively other vascular beds can be used, particularly those in the coronary circulation.\textsuperscript{36}

**EFFECT OF DISEASE ON ENDOTHELIAL FUNCTION**

The vasodilatory function of endothelial cells has an essential role in maintaining the physiological
activity of blood vessels. Disturbances in endothelial cell function decrease the release of nitric oxide and reduce vasodilation (Figure 3). Damaged or excessively activated endothelial cells secrete endothelin-1 and produce a variety of adhesion molecules and chemotactic substances. As a result, leucocytes migrate to the endothelial wall.\(^{37}\)

Near the wall they slow down and adhere to endothelial cells. This process of cell adhesion has been described over a long period of time and some of the underlying molecular mechanisms have recently been identified.

Endothelial cells induce adhesion by expressing specific surface adhesion molecules which interact with ligands on leucocytes and platelets\(^{38}\) (Figure 4). Three groups of adhesion molecules have recently been identified: a family of selectins including E selectin and L selectin, a group of integrins such as LFA-1 (leucocyte function-associated antigen) and a supergene family of immunoglobulins such as ICAM-1 (intracellular adhesion molecule) and VCAM (vascular cell adhesion molecule). These adhesion molecules are expressed after stimulation of endothelial cells by specific factors such as tissue necrosis factor (TNF-\(\alpha\)). This expression is increased on endothelial cells chronically damaged by risk factors for atherosclerosis such as smoking, hypertension and hypercholesterolaemia. In addition, high blood glucose concentrations and increased LDL cholesterol induce increased expression of ligands such as integrins which are the binding proteins for adhesion molecules.

Binding of blood cells to the endothelium affects the pathogenesis of atherosclerosis in several ways.\(^{39}\) Increased binding of leucocytes and platelets leads to changes in the laminar blood flow and to the generation of turbulence which in turn increases platelet aggregability and risk of thrombosis. The binding of leucocytes to the endothelium also affects the production and release of reactive oxygen species from these cells.\(^{40}\) In hyperglycaemic conditions binding of monocytes to glycosylated proteins can also occur.\(^{41}\)

In diabetes and hyperlipidaemia disturbed permeability of the endothelial layer results in an increased movement of substances from the circulation into the vessel wall.\(^{42}\) Insulin can migrate across the cell layer and stimulate smooth muscle cell proliferation and the mitogenic and migratory activity of other factors such as PDGF\(^{43}\) (platelet derived growth factor). In addition, endothelial cell dysfunction can lead to accelerated intravascular blood coagulation by reducing nitric oxide and prostacyclin formation and decreasing fibrinolytic activity\(^{44}\) (Figure 2).

(1) Diabetes and endothelial function

The relationship between diabetes and its vascular complications is complex but it seems likely that damage to the vascular endothelium plays a major role. Exposure of plasma and cell membrane proteins to hyperglycaemic conditions for prolonged periods results in the attachment of glucose molecules to proteins, a process known as non-enzymic glycosylation. Further slow reactions result in the formation of advanced glycosylation end-products (AGEs)\(^{45}\) which have been shown to inactivate nitric oxide and impair endothelium-dependent vasodilation.\(^{46}\) However these vascular responses are also impaired during short term hyperglycaemia suggesting that this is not the principal mechanism.\(^{47}\) In the presence of hyperglycaemia, the phytol metabolic pathway is stimulated resulting in an increased utilisation of NADPH. Since NADPH is an essential co-factor for nitric oxide synthase reduced availability
within the cell could result in reduced nitric oxide production. Depletion of NADPH could then result in enhanced oxidative stress and increased susceptibility to free radical attack. Aldose reductase inhibitors have proved effective in restoring endothelial function in experimental diabetes although their impact on the vascular complications of diabetes has been disappointing. Increased oxidative stress in diabetes results from several different sources. These include phytol pathway activity, auto-oxidation of glucose and reduced antioxidant concentrations including vitamin E and ascorbic acid. The resulting increase in free radical activity in diabetes can cause cellular damage and inactivation of nitric oxide. Several studies have demonstrated improved endothelial function in response to free radical scavengers in diabetic animals.

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Although the causes of vascular damage in diabetes are in large part due to hyperglycaemia, other metabolic factors probably contribute. Hyperlipidaemia is associated with endothelial dysfunction and increased morbidity. A large percentage of patients have lipid abnormalities including hypertriglyceridaemia, reduced HDL cholesterol, increased LDL cholesterol and increased amounts of oxidised LDL cholesterol. The role of oxidised LDL cholesterol in causing endothelial damage has recently been described in this department by measuring the cytotoxicity of LDL cholesterol from diabetic patients in cultured endothelial cells.

Hyperinsulinaemia may also be involved in producing vascular damage in diabetes and increasing the risk of developing atherosclerosis. A major hypothesis is that increased insulin concentrations occurring as a result of insulin resistance cause sodium retention and increased sympathetic activity. Both factors tend to increase blood pressure which is associated with increased cardiovascular morbidity and endothelial dysfunction in the absence of diabetes. Studies linking hyperinsulinaemia with arterial disease and hypertension are inconsistent and increased prevalence of atherosclerosis and hypertension is not a feature of insulinoma, a condition associated with extremely elevated insulin levels.

(2) Hyperlipidaemia and endothelial function

There is now a considerable amount of evidence that low density lipoproteins (LDL) impair the activity of nitric oxide. This is in part due to LDL itself but most evidence suggests that oxidised LDL has a more important and lasting effect. Endothelial cells, macrophages and vascular smooth muscle cells can oxidise LDL cholesterol. If this is not removed by antioxidants such as vitamin E it can be taken up by scavenger receptors on the macrophages where it accumulates to form foam cells and contributes to the development of atheroma. High levels of circulating LDL cholesterol have been shown to reduce endothelium-dependent vasodilator responses to aggregating platelets and prostacyclin release and could predispose to vasoconstriction, platelet adhesion and thrombus formation.

(3) Hypertension and endothelial function

Abnormalities of endothelial function are well described in hypertension. Endothelium-dependent dilation is diminished in experimental hypertension and in patients with essential hypertension. Lowering blood pressure in experimental animals prevents the development of these abnormalities but abnormal responses remain to some degree in adequately controlled hypertensive patients.

(4) Circulatory shock and endothelial function

If a little nitric oxide is good for cardiovascular health, large amounts are often dangerous. In endotoxin shock there is massive release of nitric oxide leading to vasodilation and hypotension. In animal models the blood pressure can be restored by agents such as L-NMMA which prevent nitric oxide production. Release of nitric oxide by endocardial and myocardial cells may also occur accounting in part for the depressed cardiac function which occurs in some patients with endotoxic shock. Increased nitric oxide synthesis probably also has an important role in haemorrhagic shock and we have recently demonstrated increased nitric oxide activity in patients with systemic vasculitis.

(5) Other diseases associated with abnormal nitric oxide production

Impaired nitric oxide activity in the large systemic arteries has been demonstrated in congestive cardiac failure. In the coronary circulation, in congestive cardiomyopathy and in vein grafts. Smoking is also associated with the abnormalities...
of endothelial and platelet function seen in patients with hypercholesterolaemia. In infantile pyloric stenosis a selective depletion of nitric oxide has been found in the circular pyloric muscle.

Following the identification of a nitric oxide-like factor in brain tissue in 1988 there has been extensive research on the possible role of nitric oxide as a neuronal mediator. A specific nitric oxide synthase enzyme has been purified and cloned and is most abundant in the cerebellum and olfactory bulbs. In Huntington’s disease, and in cerebral ischaemia it has been suggested that nitric oxide may protect neurones from oxidative injury. Protection, however, may depend on the concentration of nitric oxide; at low levels it appears to have an enhancing, mediating and protecting role in brain neurones while at high levels nerve damage seems to be more likely.

It has also been recently established that nitric oxide is the principal mediator of penile erection. Nitric oxide synthase is localised in pelvic neurones innervating the corpora cavernosa and in the neuronal plexuses of the adventitial layer of the penile arteries. It seems likely that erectile impotence in diabetic men involves impaired function of the L-arginine-nitric oxide pathway, and anecdotal evidence suggests that the local application of glyceryl trinitrate to the penis of impotent men causes erection with a headache in the sexual partner being the only adverse effect.

Clinical uses of nitric oxide

As discussed previously, drugs which donate nitric oxide to vascular smooth muscle and produce vasodilation have been used in clinical medicine for almost one hundred and fifty years. Glyceryl trinitrate preferentially dilates veins, a principal reason for its clinical value in angina pectoris and congestive cardiac failure. The reason for the venoselectivity could be related to the low basal output of nitric oxide, and consequently the guanylate cyclase in venous smooth muscle is upregulated and responds more readily to exogenous nitric oxide. By the same reasoning, reduced nitric oxide synthesis at sites of endothelial damage or dysfunction in arteries or arterioles should make these vessels more sensitive to the actions of nitrodrilators. The antiplatelet effects of nitric oxide provided by organic nitrates may also have some beneficial therapeutic effects. Recent studies have focused on the development of new classes of nitrovasodilators. S-nitrosogluthathione (GINO) has recently been shown to have selective antiplatelet effects.

New uses for nitric oxide are being explored. These include the local application of organic nitrates for the treatment of impotence, systemic administration for acute stroke and delayed labour and inhalation of nitric oxide in a variety of respiratory conditions such as respiratory distress syndrome, acute lung injury, pulmonary hypertension and in diseases associated with ventilation-perfusion inequality.

There have been a number of encouraging reports on the value of inhaled nitric oxide in pulmonary hypertension. The condition is frequently observed in neonatal and in adult respiratory distress syndrome and following cardiopulmonary bypass surgery. However, inhaled nitric oxide remains an experimental form of treatment and there is no useful information on the benefits of nitric oxide in any chronic respiratory disease. Most chronic lung diseases are associated with structural changes so that the effects of inhaled nitric oxide are unlikely to result in major benefit. Inhaled nitric oxide is not without its own problems. Nitric oxide can combine with oxygen resulting in nitric acid formation and increased toxic radicals. Methaemoglobinemia can occur and at high concentrations it could exert pro-inflammatory effects.

Other drugs may increase the production of endogenous nitric oxide. Angiotensin converting enzyme inhibitors inhibit the breakdown of bradykinin which in turn stimulates the release of nitric oxide from the endothelium in patients with coronary artery disease and congestive heart failure. Recent interest has focused on the possibility of enhancing nitric oxide production by providing excess of its substrate L-arginine. Although arginine is plentiful in the diet and is not the rate limiting factor for nitric oxide synthesis, there are reports that arginine prevents the onset of atheroma in experimental animals and improves endothelial function in patients with hypercholesterolaemia, and in patients following cardiac transplantation. In a recent study involving patients with heart failure, chronic L-arginine supplementation was found to improve blood flow.
Clinical methods and indications for the reduction of nitric oxide

In some clinical situations it may be valuable to inhibit the synthesis or activity of nitric oxide. Inhibition of nitric oxide synthesis with substrate analogues such as NO\(^\text{NO}^+\) monomethyl-L-arginine reverses the local vasodilation associated with inflammation and the profound hypotension and cytotoxic effects which occur in endotoxin shock.\(^{59}\) Selective inhibitors of the inducible isoform of nitric oxide synthase are being developed as possible anti-inflammatory drugs. Such agents would have the theoretical advantage that they would inhibit the pathophysiological production of nitric oxide without impairing endothelial, neuronal or platelet function. Drugs in common clinical use may also reduce nitric oxide synthesis. Corticosteroid drugs inhibit the induction of nitric oxide synthase but are ineffective once the enzyme is expressed. Methotrexate inhibits the synthesis of tetrahydrobiopterin, an essential cofactor for the induction and activity of nitric oxide synthase.\(^{76}\) The relationship between this and the drug’s clinical effect is unknown but the findings raise the possibility of developing more specific anti-inflammatory or immunosuppressive drugs based on alteration of the inducible nitric oxide pathway.

Nitrate tolerance

Although nitric oxide is the active component of drugs collectively known as the nitrovasodilators some are able to release nitric oxide directly while others require metabolic conversion by muscle cells. Sodium nitroprusside spontaneously releases nitric oxide from its complex.\(^{77}\) Organic nitrates such as glyceryl trinitrate, isosorbide dinitrate and isosorbide mononitrate require the presence of sulphydryl (SH) groups within the cells to release and generate nitric oxide\(^{78}\) (Figure 1). With continuous administration of organic nitrates tolerance to their effects in angina and heart failure has been described especially if the transdermal route is employed.\(^{79,80}\)

Tolerance to the vascular effects of organic nitrates has long been recognised in the munitions industry.\(^{81}\) The headaches, flushing and lightheadedness experienced during early exposure became less intense or disappeared when workers had been exposed for several days. Such tolerance was short lived and when workers were away from the factory for short periods symptoms recurred on re-exposure. In addition, Lang and colleagues 1972 described chest pain in munition workers during periods away from work which were thought to be due to coronary vasoconstriction.\(^{82}\) Current concepts of nitrate tolerance have focused on impaired intracellular metabolic conversion of nitrate to nitric oxide. When tolerance develops there appears to be a decreased ability of the organic nitrate to undergo metabolic conversion to nitric oxide probably due to a relative unavailability of reduced intracellular thiol groups.\(^{83}\) Recent evidence suggests that extracellular pathways of organic nitrate metabolism may provide an alternative pathway for conversion to nitric oxide.\(^{84}\) In the tolerant state, there is reduced nitric oxide generation leading to failure of smooth muscle relaxation.

In vitro experiments have documented decreased generation of cyclic guanosine monophosphate in the presence of nitrate tolerance. There appears to be no abnormality of the enzyme guanylate cyclase however which remains fully responsive to sodium nitroprusside and bypasses the cysteine-dependent metabolic cascade. Several studies have demonstrated that sulphydryl donor administration with N-Acetylcysteine or methionine can reverse or prevent tolerance which occurs during sustained nitrate administration.\(^{85}\) The value of this type of treatment, in preventing nitrate tolerance is at present of little practical value.

INTERVAL THERAPY FOR NITRATE TOLERANCE

A number of investigations have demonstrated that nitrate dosage regimens, punctuated by prolonged nitrate-free intervals, can maintain the beneficial clinical effects of nitrates in angina pectoris\(^{86,87}\) and congestive cardiac failure.\(^{88,89}\) Overall, most studies support the view that the use of intermittent nitrate therapy is less likely to result in tolerance than continuous therapy administered by the oral,\(^{90}\) intravenous,\(^{85}\) or transdermal\(^{91}\) routes. While maintaining a dosage-free interval of at least eight hours has been successful in reducing the incidence of nitrate tolerance, the approach has a number of potential disadvantages. The main problem is a worsening of the chest pain during the period when the drug is withdrawn. Fortunately the adverse consequences of intermittent therapy have been minimal in most studies. However in a large placebo-controlled study intermittent transdermal...
glyceryl trinitrate therapy produced nocturnal or rest angina during the drug free interval in the active treated group but not in the placebo group. The possibility of glyceryl trinitrate rebound phenomenon must be kept in mind in view of the previous observations experienced by munition workers.

Studies of silent myocardial ischaemia in coronary heart disease have demonstrated a low frequency of ST segment depression in the period from midnight to 7 am in patients with chronic stable exertional angina. This supports the view that the usual nitrate-free period during sleep causes problems in patients with stable angina without rest or nocturnal pain. The use of other antianginal drugs such as beta adrenoceptor antagonists and calcium channel antagonists is clearly an important strategy in providing protection against ischaemia during the nitrate-free interval.

Nitrate Resistance

A variety of cardiovascular conditions are associated with primary nitrate tolerance or resistance. Failure to reduce blood pressure with intravenous doses of glyceryl trinitrate in excess of 200 μg/min is commonly seen in patients with congestive cardiac failure. Possible mechanisms include primary receptor resistance and secondary mechanisms such as catecholamine-induced increases in vaso-constrictor tone, activation of the renin angiotensin system, haemodilution, impaired dilation due to vessel wall oedema and impaired hepatic metabolism of the nitrate. Impaired or absent haemodynamic responses are also occasionally observed in the period following an acute myocardial infarction. Failure of the blood vessels to dilate suggests primary receptor tolerance which is not overcome by very high doses. In a series of experiments we demonstrated impaired vasodilator responses to glyceryl trinitrate in patients with non-insulin dependent diabetes mellitus when compared with age and sex matched controls. This impaired response was somewhat surprising since diabetes is associated with impaired endothelial function and nitrate-induced vasodilation has been shown to be increased when the endothelium is damaged. I have already discussed that the biotransformation of organic nitrates requires intracellular sulphhydryl groups to produce vasoactive intermediates which stimulate guanylate cyclase. Oxidation or depletion of these sulphhydryl donors will lead to impaired responsiveness to organic nitrates. In diabetes antioxidant activity is decreased. Increased oxidative stress and enhanced free radical activity occurring in diabetes probably alters the oxidation-reduction equilibrium of intracellular thiols and results in primary oxidation or depletion of the essential sulphhydryl donors. This seems the most likely explanation for the impaired responses observed in diabetes. In conclusion, resistance to the action of organic nitrates has been demonstrated in congestive cardiac failure, type II diabetes and following an acute myocardial infarction. Studies performed in our laboratory would also suggest that a significant percentage of normal subjects show impaired vasodilator responses to organic nitrates.

CONCLUSIONS

Although nitrates have been prescribed for patients with angina pectoris for over a century and more recently for congestive cardiac failure, it is only over the last few years that new insights into their mode of action have been identified. The discovery of the endogenous nitrovasodilator nitric oxide, produced in endothelial cells by the enzyme nitric oxide synthase has greatly expanded our knowledge of the action of organic nitrates. Nitric oxide, when released from endothelial cells can interact with vascular smooth muscle and platelets by activating soluble guanylate cyclase and increasing cyclic GMP. In vascular smooth muscle this causes vasorelaxation and reduced platelet adhesion. Exogenous nitrodilators exert their action by producing nitric oxide. Their vasodilator activity is greatest in blood vessels with low basal production of nitric oxide such as veins and vessels in which the endothelium is removed or damaged. A significant percentage of patients with heart failure, diabetes and ischaemic heart disease fail to respond to nitrate therapy however. Long term use of organic nitrates is associated with the development of tolerance or of reduced therapeutic efficacy when administered in doses or formulations which maintain therapeutic plasma levels over a twenty four hour period. The administration of N-acetylcysteine, angiotensin converting enzyme inhibitors or diuretics do not consistently prevent nitrate tolerance. At present intermittent therapy is the only satisfactory method to reduce nitrate tolerance although other antianginal drugs may be required during the nitrate-free intervals.

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