Heart International / Vol. 3 no. 3-4, 2007 / pp. 112-121 © Wichtig Editore, 2007

Organic nitrates and tolerance in cardiovascular diseases: effects of isosorbide-2-mononitrate compared to traditional nitrates

RICCARDO RADDINO, GIORGIO CARETTA, DEBORA ROBBA, GREGORIANA ZANINI, IVANO BONADEI, ALBERTO MADURERI, LIVIO DEI CAS

Section of Cardiovascular Diseases, Department of Experimental and Applied Medicine, University of Brescia, Operative Unit of Cardiology - "Spedali Civili" Hospital, Brescia - Italy

ABSTRACT: Nitrates are a cornerstone in the treatment of coronary heart disease and heart failure. Although they are widely used in clinical practice, their therapeutic value is partially compromised by the rapid development of tolerance. The effect of different nitrates, nitroglycerin (NTG), isosorbide dinitrate (ISDN), 5-mononitrate (5MN) and 2-mononitrate (2MN) and the mechanism responsible for nitrate tolerance were investigated on isolated rabbit hearts and aortic strips. Preparation was stimulated by different spasmogenic agents: KCl, angiotensin II (A) and noradrenaline (NA); nitrates were administered on the plateau contraction, at the concentration of maximum inhibitory effect. Nitrates produced the following maximum inhibitions on NA-induced contraction: NTG 90% (10^{-6} M), ISDN 60% (10^{-4} M), 5MN 55% (10^{-4} M) and 2MN 80% (10^{-4} M). In another series of experiments, preparations were pre-incubated with the maximum inhibitory concentration of each nitrate to evaluate the induction of tolerance. After incubation a loss of vasodilator effect of nearly 50-60% was observed for all the nitrates considered except 2MN, in which the loss of effect was significantly lower (36%). The cyclic GMP (cGMP) levels measured in the preparations were lower in the presence of 2MN than in the other compounds. These data suggest that 2MN is able to induce a lesser cGMP increase and a lesser tolerance induction; since these observations seem to be correlated the vasodilator effect of 2MN probably involves mechanisms other than guanylyl cyclase stimulation. (Heart International 2007; 3: 112-21)

KEY WORDS: Nitroglycerine, Tolerance induction, Nitric oxide, Isosorbide mononitrate

INTRODUCTION

Nitrates have been widely used in cardiology for over 100 yrs. These drugs are excellent agents for the treatment of coronary syndromes (namely stable angina, unstable angina and myocardial infarction) and patients with chronic congestive heart failure (HF) or acute HF.
Their leading hemodynamical effect is the reduction of cardiac preload by venodilation and an increase in venous capacitance. Nitrates also induce the vasorelaxation of both normal and stenotic coronary arteries and coronary collateral vessels; therefore, preventing episodic coronary constriction. They have been shown to reduce left ventricular diastolic pressure, left ventricular volume and mitral regurgitation. Finally, antiplatelet and endothelial protective actions have been suggested (1).
The nitrate compounds most commonly used in clinical practice are nitroglycerin (NTG), isosorbide-dinitrate (ISDN) and isosorbide-5-mononitrate (5MN). They are available in different dosage forms including sublingual, intravenous (i.v.), oral and transdermal preparations (Tab. I). Rapid and short-acting preparations are excellent in treating acute coronary syndromes or angina at-

1826-1868/112-10$25.00/0

TABLE I - NITRATE PREPARATIONS, ROUTES OF ADMINISTRATIONS AND PHARMACOKINETICS

Drug and route	Onset of action (minutes)	Duration of action (hours)
Sublingual		
- NTG	2-5	1-2
- ISDN	5-7	2-3
Oral		
- ISDN	30	3-6
- 5MN	30	3-6
- ISDN, 5MN (SR)	60-120	12-16
Transdermal		
- ISDN	30-60	3-24
- NTG	30-60	12-16
Intravenous - NTG, ISDN	1-2	During infusion and 30 min after

NTG: nitroglycerin; ISDN: isosorbide-dinitrate; 5MN: isosorbide-5-mononitrate; SR: sustained release

tacks; long-term chronic administration is useful in treatment of silent or symptomatic ischemic heart disease and HF.

Mechanisms of action

The organic nitrates are pro-drugs and need to be enzymatically degraded to produce their effects. Once adsorbed, they undergo biological transformation in live and vascular smooth muscle cells (SMCs) yielding nitric oxide (NO). The bioactivation involves P450 enzyme(s) in the smooth endoplasmic reticulum.

NO in an important factor physiologically produced in many tissues and involved in several biological roles. Endogenous NO was originally identified as endothelium-derived relaxing factor (EDRF). It exerts vasodilatation effects, reduces platelet aggregation and controls endothelial function. NO acts stimulating guanylyl cyclase leading to the formation of cyclic GMP (cGMP) from guanosine triphosphate. cGMP leads to the relaxation of SMCs through the activation of specific cGMP-dependent kinases and the phosphorylation of several proteins. This biochemical cascade brings about the inhibition of calcium influx into the cell (2) and the dephosphorylation of myosin light-chains, preventing actin-myosin interaction.

Recently, it has been proposed that NO could also activate, directly or through a second messenger, the potassium channels on SMC membrane, determining hyperpolarization and consequent relaxation.

Finally, recent studies have discovered an alternative metabolic pathway for NTG (but not for other nitrate compounds) that seems to be the most active at therapeutic dosages. This pathway involves mitochondrial enzymes and the respiratory chain to yield NO or a related species (NO_X) (3). These findings could partially explain the difference in both potency and tolerance induction of NTG in comparison with other compounds.

Tolerance development

A crucial problem in the clinical use of nitrates is the tolerance phenomenon. Tolerance is defined as a condition in which the body becomes accustomed to a drug, so that the previous dose no longer produces the previously obtained effect and progressively larger doses are needed. Since the therapeutic properties of nitrates were discovered, it was soon recognized that long-term treatments resulted in tolerance development. In managing coronary heart disease, the attenuation or loss of one or several of the beneficial effects of these drugs has frequently been observed. All regimens using frequent doses of long-acting nitrates (more than twice daily) or continuous delivery systems (patches, continuous i.v. infusion) will rapidly develop tolerance, often occurring within 24 hr (4).

Several mechanisms have been proposed to explain nitrate tolerance such as sulfhydryl donor depletion, neurohormonal activation or intravascular plasma volume expansion.

Depletion of sulfhydryl groups

Depletion of intracellular sulfhydryl groups has widely been considered one of the most important causes of nitrate tolerance development. Sulfhydryl groups are needed for the conversion of nitrates to NO in vascular microsomes. This hypothesis was supported by animal and clinical studies showing that the administration of relatively large doses of cysteine or N-acetylcysteine (critical intracellular sulfhydryl co-factors) could prevent or reverse the tolerance to the vasodilator action of repeated NTG administration (5).

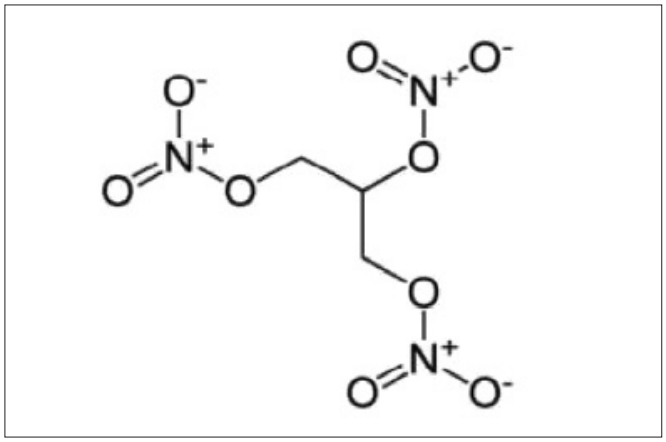

Fig. 1 - *Molecular structure of NTG.*

Neurohormonal activation and plasma volume expansion

Another propounded theory involves the activation of neurohormonal mechanisms such as the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS). The persisting reduction of preload and blood pressure (BP) after sustained nitrate administration stimulates a compensatory activation of both these systems. Moreover, a drop in renal blood flow was suggested to be a further mechanism contributing to RAAS activation; this stimulation has been observed especially in patients with HF treated with nitrates. Long-lasting NTG treatment as been associated with increases in plasma renin activity. These responses may act to attenuate or reverse the hemodynamical effects of nitrates. Many authors defined all of these neurohormonal adjustments as ''pseudotolerance" (6).

Venous dilation induced by nitrates brings about a reduction in the capillary hydrostatic pressure and major water retention in the intravascular space. This leads to hemodilution, plasma volume expansion and a rise in BP. According to this hypothesis hematocrit reductions have been observed after nitrate treatments (7).

Free radical production

Recently, a theory involving endothelial molecular mechanisms was proposed to explain tolerance. Studies demonstrated that nitrate treatments are associated with increased superoxide production by the endothelium (8-10). It has been observed that superoxide in endothelial and smooth muscle vascular cells reacts with NO, generating peroxynitrite. Studies suggested that the mechanism underlying the development of tolerance was the sequestration of NO by superoxide free radicals (3). These observations are supported by several studies suggesting that the concurrent administration of antioxidants (such as vitamins C and E, ebselen) could lead to a reduction in nitrate tolerance.

Relationship between the mechanism of action and nitrate tolerance induction of different nitrates

Introduction

Studies concerning the action of NTG (Fig. 1) and other nitroderivates pointed out that these substances induce vasodilatation through the activation of specific ''sites" in vascular cells (2). Two different locations of these sites have been proposed, namely the endothelial and/or the SMC plasma membranes; moreover, two corresponding post-receptor events have been suggested to play a role. The first is consistent with a release of inhibitory prostanoids (PGF2 , PGE2) from the endothelium (11, 12), while the second with a cGMP accumulation in SMC through the activation of the guanylate cyclase on the cell membrane (13, 14). However, some studies have challenged the possible involvement of prostaglandins. Despite the ability of cultured endothelial cells to produce prostacyclin in response to NTG, the blockade of cyclooxygenase with indomethacin does not modify vasodilator activity of NTG in coronary vessels *in situ* (15, 16).

Contrarily, a large increase of cGMP levels was demonstrated in vascular SMCs in response to different nitroderivatives, including NTG (17-19), ISDN (20) and 5MN (21); therefore, suggesting a direct involvement of the nucleotide as a peculiar second messenger, but not exclusive (22) to this class of drugs. Biochemical events leading to the rise of cGMP levels involve the formation of NO or nitrothiols (RSNO) in the presence of sulfhydryl groups (SH). Both NO and RSNO stimulate guanylate cyclase to produce cGMP which, in turn, limits calcium availability in the intracellular spaces (17) and interferes with the activation of the contractile system (23). As previously noted, the key role of SH groups in cGMP synthesis was emphasized in order to explain a possible

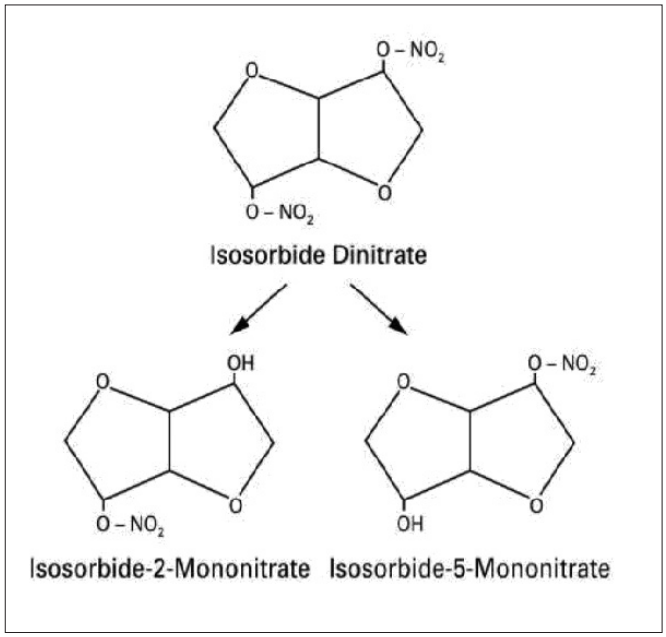

Fig. 2 - *Molecular structure of ISDN, 5MN and 2MN.*

mechanism of the nitrate tolerance phenomenon.
Although several mechanisms of tolerance induction have been suggested, new compounds are synthesized in an attempt to limit tolerance development and, at the same time, to obtain longer acting drugs. An example is isosorbide-2-mononitrate (2MN) that differs from ISDN for the absence of NO_2 group in position 5 (Fig. 2).
The vasodilator activity of 2MN was evaluated on different isolated preparations in comparison with other compounds most commonly used in clinical practice, namely NTG, ISDN and 5MN. Isolated rabbit hairs and aortic strips were used as experimental models to evaluate drugs acting on coronary resistances and vascular contractility.

Materials and methods

White New Zealand rabbits of both sexes weighing 2-2.5 kg were used. The animals were sacrificed by cervical dislocation. The hearts were quickly removed and placed into an ice-cold Ringer-Lock solution, oxygenated with 100% O_2 and containing in mM: NaCl 136.9, KCl 2.68, $MgCl_2$ 1.7, $NaHPO_4$ 0.42, $NaHCO_3$ 3.93, and glucose 5.55 (pH 7.4). Immediately after the removal of the heart, the thoracic segments of the aorta were submerged and placed in modified Krebs-Henseleit solution containing in mM: NaCl 113, KCl 4.7, $CaCl_2$ 1.9, $NaHCO_3$ 25, $MgSO_2$ 1.2, KH_2PO_4 1.2 and glucose 11.5. The solution was bubbled with 95% O_2 and 5% CO_2 (pH 7).

Heart preparations

A previously described procedure was followed (24). After the removal of the pericardium and surrounding tissues, the hearts were perfused with Ringer-Locke solution according to the non-recirculating Lagendorff technique (24). The perfusion fluid was continuously gassed with 100% O_2, maintained at 37 °C and delivered to the aortic inflow cannula at a constant rate of 22-24 ml/min using a peristaltic pump (Gilson, Miniplus HP2HF, Villers - Le Bel, France). The perfusion pressure was measured by a Statham transducer connected to the side arm of the perfusion cannula. Since retrograde flow (coronary flow) was held constant during the experiment, the coronary perfusion pressure represented a direct measure of coronary resistance. A fluid filled balloon connected to a pressure transducer was inserted into the left ventricular cavity through an opening in the left atrium; therefore, obtaining an isovolumically beating preparation (25). Both left ventricular pressure (LVP) and coronary perfusion pressure (CPP) were simultaneously recorded by a polygraph (ITE Biomedica, Florence, Italy). With the exception of those experiments in which the chronotropic effect was evaluated, the hearts were electrically paced in order to exclude LVP variations due to heart rate (HR) changes. Rectangular pulses (1 ms width at 0.5 V up to threshold stimulation) were applied to the preparation through two platinum electrodes, one connected to the metal inflow of the cannula and the other directly in the ventricular apex. The stimulation frequency was 10% greater than the basal HR. Hearts were left to equilibrate for 30 min before the administration of drugs. Drugs were administered into the perfusion fluid and allowed to act until the maximum effect was obtained. (5-15 min). NTG, ISDN, 5MN and 2MN were dissolved in distilled water and administration was performed according to the cumulative doses method (26).

Aorta preparations

The media was then separated from the connective tissue and from the adventitia, spirally cut strips of thoracic aorta were set up, according to the Furchgott technique (27). Spirally cut strips (2 cm long, 3 cm wide) were placed in 10 mL of organ bath at 37 °C containing

Krebs-Henseleit solution, composition as described above. Contractions were measured by an isometric transducer connected to a pen-writing recorder (Unirecord, Basile, Milan, Italy). An initial tension of 2 x 10^{-3} kg was applied for 120 sec before drug administration. Spasmogenic compounds, KCl (10^{-1} M) and angiotensin II (10^{-6} M) were administered directly into the organ bath. When the contractions reached a plateau (usually 5-10 min after administration) the nitrates were administered according to the cumulative doses method (28). Some aortic strips were rubbed to eliminate the endothelial function. The ablation of the endothelial structure was pharmacologically verified by the acetylcholine test (29, 30) and the inhibitory effect of the nitrates was then evaluated, as described for non-rubbed preparations.

Induction of tolerance

Aortic strips were left to incubate for 120 min in the presence of a nitroderivate concentration able to exert the maximum inhibitory effect on the noradrenaline (10^{-6} M) induced contraction. Preparations were then washed and a contraction with noradrenaline was induced. Inhibitory activity of the nitroderivates was then determined and compared with the inhibition obtained in the control experiment.

Cyclic-GMP measurement

Cyclic GMP content was determined in a series of aortic strips set up as described above. The nucleotide levels were measured both in control experiments, where only the stimulant (KCl, noradrenaline or angiotensin II) was administered to the organ bath, and in the presence of different nitrates, administered at the top of the plateau elicited by the stimulant and left acting for 2 min. At this time, preparations were frozen with a pre-cooled clamp and put in liquid nitrogen; a standard procedure was followed (31). Frozen strips were then homogenized in 1 mL of 6% trichloroacetic acid and centrifuged at low speed for 20 min. The supernatant fractions were extracted four times with 5 mL of water-saturated diethyl ether.

The aqueous extract was dehydrated at 60 °C under a stream of nitrogen. Residual materials were reconstructed with sodium acetate buffer and assayed by a radioimmunoassay kit (Amersham, USA), to evaluate the cyclic GMP content.

Data evaluation

Results are expressed as mean ± SEM of six-eight independent experiments. Inhibitory responses on basal LVP and CPP were calculated as a percentage of inhibition, while coronary spasm as a percentage of inhibition on the CPP increases. Statistical analysis was performed by analysis of variance and test of simple main effect. Student's t-test was performed for the comparison of maximum effects of calcium curves. The level of significance was considered as $p<0.05$.

Drugs

The following drugs were used: nitroglycerin (Simes), isosorbide dinitrate (Wyeth), isosorbide-5-mononitrate (Chiesi Farmaceutici), isosorbide-2-mononitrate, vasopressin (Sandoz), noradrenaline (Merck), angiotensin II (Hypertensiva Ciba).

Results

Heart

Thirty minutes after set-up, isolated rabbit hearts developed a basal LVP value of 58.9 ± 5.1 mmHg and basal CPP of 68.3 ± 3.8 mmHg. In control group experiments, variations <10% of these values were measured in the following 3 hr. Heart rate was 148 ± 7 bpm at the beginning of the experiment and then spontaneously decreased to 115 ± 5 bpm.

Vasopressin 10^{-9} M induced a long-lasting coronary spasm, being able to increase CPP of 71.5 ± 4.7 mmHg. An erratic reduction of LVP was sporadically observed.

Effects of nitroderivates

NTG and other compounds did not substantially modify basal CPP, LVP and HR up to 10^{-4} M.

When administered in the presence of a vasopressin induced spasm they induced a prompt vasodilator effect. Vasodilation was different according to different compounds.

NTG (10^{-7} M - 10^{-4} M) and 2MN (10^{-5} M and 10^{-4} M) resolved vasopressin induced spasm, while 5MN and ISDN were less potent and effective. We considered the effects obtained with concentrations of 10^{-5} M to give an idea of these differences (Fig. 3).

It was evident that NTG was the more effective compound, being able to reduce coronary spasm by 80%, while 2MN was actually more active than 5MN and ISDN.

Aorta

As already observed in isolated hearts, not all nitroderivates modified the basal tone of the rabbit aorta strips. When SMC contraction was induced by KCl or angiotensin II, a marked vasodilator effect was obtained. NTG was the most active compound in inhibiting KCl-induced aortic contraction (10^{-8} - 10^{-5} M). ISDN and 2MN were virtually equiactive, being able to inhibit KCl-induced spasm in the same range of doses (10^{-8} - 10^{-4} M) and to the same extent (60%). 5MN was the less effective compound (Fig. 4a).

The angiotensin II-induced spasm was highly sensitive to the inhibitory action of all nitroderivate drugs used (Fig. 4b), although NTG and ISDN were the more active compounds (10^{-9} and 10^{-7} M).

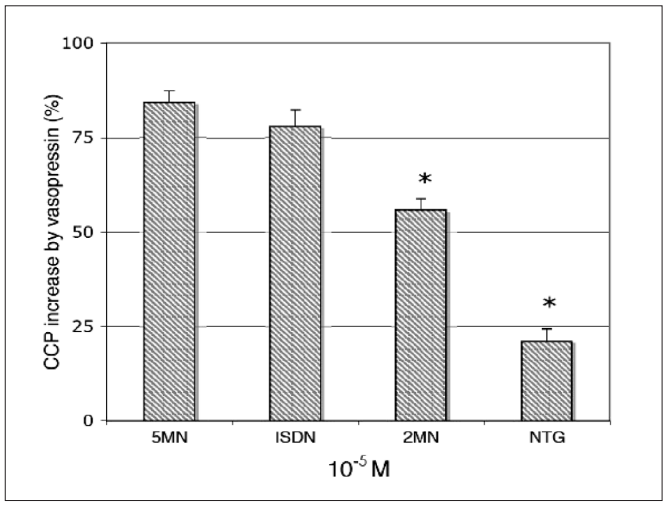

Fig. 3 - *Rabbit heart: effects of NTG, ISDN, 5MN and 2MN at the same concentration (10^{-5} M) in the vasopressin induced coronary spasm.*

Endothelium ablation did not modify the spasmolytic activity of the different drugs tested in this study (Fig. 5).

In an attempt to correlate spasmolytic activity with guanylate cyclase stimulation, we evaluated cGMP levels both in basal conditions and in the presence of nitrates (Tab. II).

Preparations were contracted with KCl, noradrenaline and angiotensin II, a nitroderivative dose, known to produce the maximum inhibitory effect was administered on the plateau of the contraction. In basal conditions, no differences in the cGMP levels were measured, comparing data obtained in the presence of the three stimulants. The extent of the vasodilator effect of the nitroderivates proved to be unrelated to the respective cGMP levels, which were greater in the case of NTG than in the other compounds. The maximum cGMP induced by 2MN was equivalent to the one induced by ISDN and 5MN, but it was lesser if considered equiactive to spasmolytic concentrations (Tab. II).

Induction of nitrate tolerance elicited a loss of activity of all nitroderivates, as seen in Figure 6 for NTG. However, the degree of desensitization was significantly lower in the case of 2MN (Fig. 7).

Discussion

Results obtained in this study suggest that all nitroderivates tested were able to induce a vasodilator effect in coronary and aortic smooth muscle, even though they differed in their activity and efficacy.

Considering the coronary district of the isolated rabbit heart, the vasodilator effect of nitroderivates is virtually undetectable on basal CPP, while it is particularly evident when coronary tone is pharmacologically increased by vasopressin. NTG represented the most po-

TABLE II - RABBIT AORTIC-STRIPS

	KCl		Noradrenaline		Angiotensin II	
	cGMP	Inhibition	cGMP	Inhibition	cGMP	Inhibition
Control	61	0	76	0	79	0
NTG	264	90%	285	90%	240	100%
ISDN	141	60%	156	65%	122	100%
5MN	157	25%	212	55%	161	100%
2MN	158	70%	171	80%	135	100%

cGMP content (pmol/g) and degree of inhibition of aortic contractions elicited by different stimulants (% of maximum effect) measured in basal conditions (control) and in the presence of nitroderivates. Doses of nitroderivatives are expressed in molar concentrations. Standard errors are less than 10% of the respective values

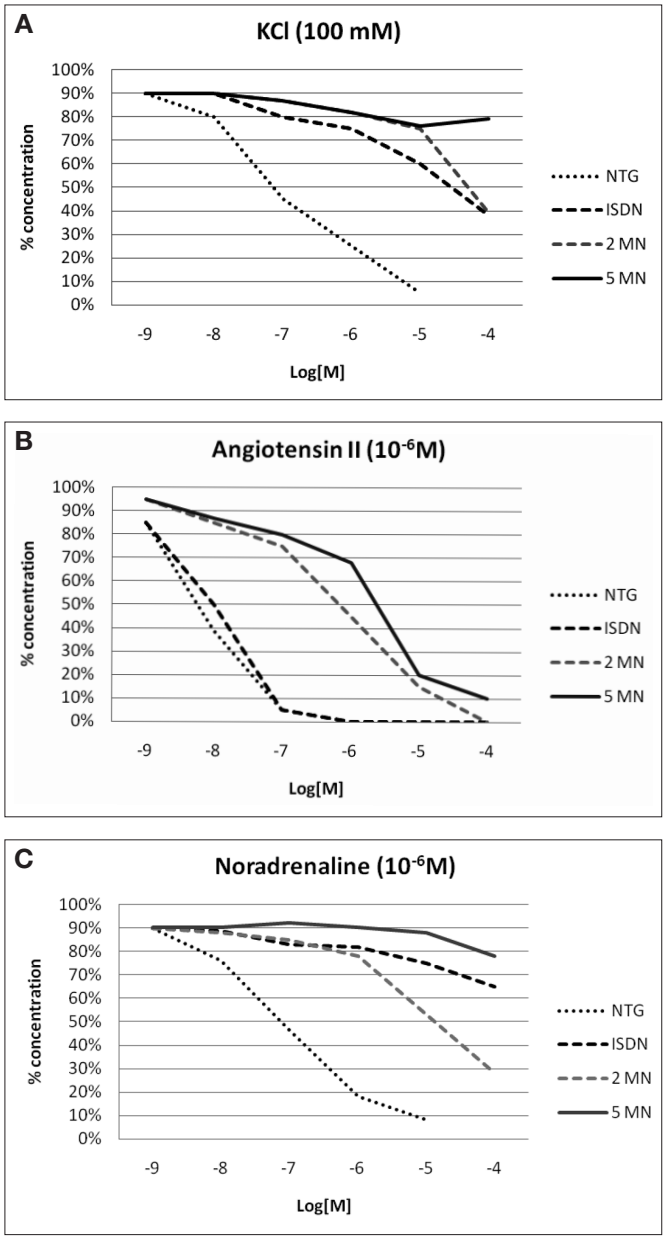

Fig. 4 - *Inhibitory effects of nitroderivates on spasm induced by KCl (a), angiotensin II (b) and noradrenaline (c). In abscissa: doses of nitrates expressed in molar concentrations. In ordinate: percent of residual contraction.*

tent and efficacious compound, being able to induce a decrease of 70% on vasopressin induced spasm. In comparison, 2MN, 5MN and ISDN were significantly less effective. These limited effects of compounds other than NTG are deemed of interest, especially in considering the high use of ISDN and 5MN in clinical practice (32, 33). As previously noted, recent studies have described an alternative metabolic pathway for NTG involving mitochondrial enzymes and respiratory chain that could explain the greater potency of this drug when compared to other nitroderivates (3).

However, some conclusions should be drawn. The stimulus used to contract the coronary bed (ie vasopressin) probably does not represent a physiological pattern in experimentally reproducing the coronary spasm as it occurs in humans; therefore, a direct correlation with clinical results cannot be done.

Furthermore, the CPP increase is the result of the total effect of vasopressin both in the epicardial and in the intramyocardial coronary vessels. Since intramyocardial vessels are probably more important for the maintenance of coronary pressure in the retrograde-perfused rabbit heart, a different selectivity of nitroderivates for different coronary districts could also be considered; other studies seem to suggest this (34, 35). Whether this presumed selectivity is due to different calcium pools involved in the excitation-contraction coupling in large vs. small caliber vessel SMCs (36) or it arises from a peculiar distribution of nitrate action sites is matter of speculation. On the other hand, the coronary activity of 2MN, although of lower efficacy compared to NTG, is deemed of interest because it has been obtained in the complete absence of inotropic and chronotropic effects; in addition, the potential pharmacokinetic advantages when this drug is given to patients in clinical practice should also be considered. Mononitrate forms of isosorbide have 100% bioavailability and 2MN is the most active one: the minimum critical plasma concentration for therapeutic effect in vivo is approximately 100 ng/ml for 5MN and approximately 20 ng/ml for 2MN.

Aortic SMC preparations are a more suitable tool to define the site and the mechanism involved in the vasodilation. The contractile activity can be induced by different stimulants able to act and to interfere with rather specific calcium sources (intra- and extracellular).

In these preparations, NTG is again the most effective compound among the nitroderivatives tested, being able to completely inhibit the contraction induced by both KCl and angiotensin II. The action of NTG resulted in being non-specific, and in being unable to distinguish between extracellular (KCl) and intracellular (angiotensin II) mediated contractions.

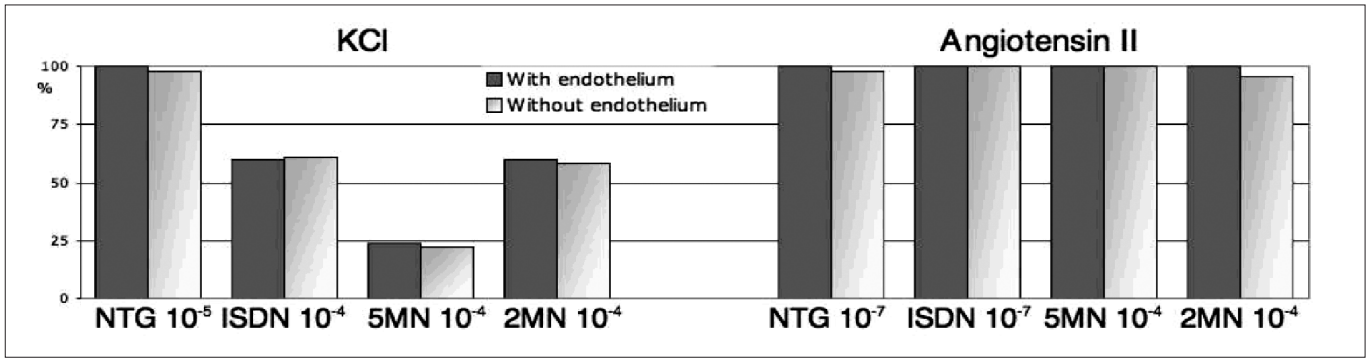

Fig. 5 - *Rabbit aorta strips: percent of inhibitory effect of nitroderivatives tested at the maximum effective concentrations, on KCl (and angiotensin II) induced contractions in preparations with or without endothelium. Doses are expressed in molar concentrations. Standard errors are less that 10% of respective value.*

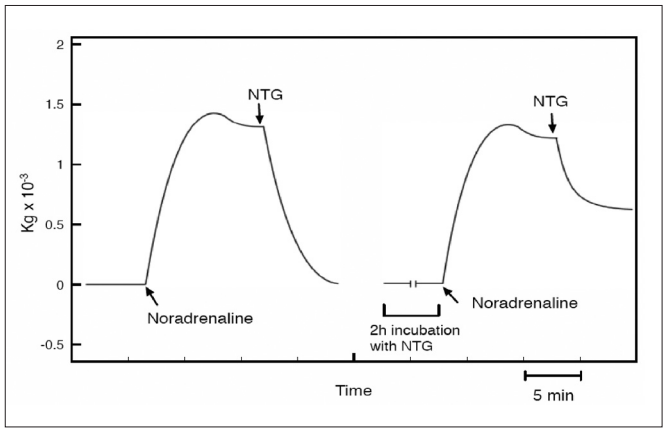

Fig. 6 - *Aorta rabbit strips: original experiment showing NTG-induced tolerance in a preparation pre-incubated with the same NTG for 2 hr.*

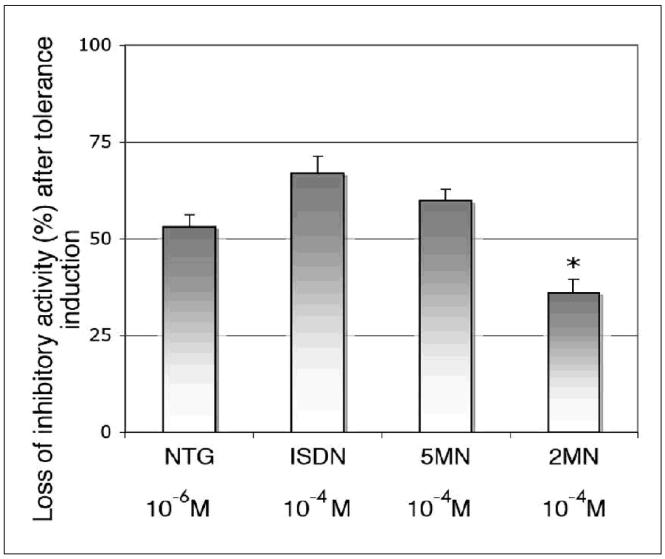

Fig. 7 - *Lack of inhibitory activity of NTG, ISDN, 5MN and 2MN after induction of tolerance as represented in Figure 6.*

On the contrary, other nitroderivates used, showed a greater level of inhibitory activity when tested on angiotensin-induced contractions; the inhibition on KCl-induced contractions was limited except for ISDN. These results suggest that isosorbide mono- a dinitrate compounds preferentially interfere with intracellular calcium availability, in a site apart from the membrane calcium channels.

The measurement of cGMP reveals that all these drugs increase the cellular content of this nucleotide; therefore, suggesting that the intracellular event leading to the inhibition of contractility is induced through guanylate cyclase stimulation.

However, no relationship between the amount of measured cGMP and the extent of inhibitory activity was observed. This fact seems to contrast with the most commonly accepted theories concerning nitrates mechanism of action (13, 14). In spite of that, a dissociation of cGMP levels and vasodilation has already been indicated in other studies using NTG and other nitrates in rat aorta (19).

Based on these results, it could be speculated that the amount of cGMP increase is peculiar for each nitroderivative, and it depends on different mechanisms involved in vasodilatation. Different metabolic pathways and pharmacodynamics could coexist contributing to different vasodilatation effects.

These hypotheses could also explain the differences

observed regarding the development of tolerance. In our experiment, tolerance development seemed to be related to cGMP accumulation, being greater in the case of NTG; therefore, confirming that SH group consumption is an important molecular mechanism responsible for this phenomenon. This reinforces the concept that alternative mechanisms could be operative in the case of 2MN, which induces a lesser degree of tolerance and a lesser increase of cGMP.

These findings, associated with a growing number of recent observations, support the concept that nitrovasodilators do not represent a homogeneous class of drugs.

Address for correspondence:
Prof. Riccardo Raddino
Section of Cardiovascular Diseases - Department of Applied Experimental Medicine, University of Brescia
Operative Unit of Cardiology
"Spedali Civili" Hospital, Brescia - Italy
riccardo.raddino@libero.it

REFERENCES

1. Loscalzo J. Antiplatelet and antithrombotic effects of organic nitrates. Am J Cardiol 1992; 70: 18B-22B.
2. Lincoln TM, Komalavilas P, Cornwell TL. Pleiotropic regulation of vascular smooth muscle tone by cyclic GMP-dependent protein kinases. Hypertension 1994; 23: 1141-7.
3. Kleschyov AL, Oelze M, Daiber A, et al. Does nitric oxide mediate the vasodilator activity of nitroglycerin? Circ Res 2003; 93: e104-12.
4. Steering Committee Transdermal Nitroglycerin Cooperative Study. Acute and chronic antianginal efficacy of continuous twenty-four-hour application on transdermal nitroglycerin. Am J Cardiol 1991; 68: 1263-73.
5. Packer M, Lee WH, Kessler PD, Gottlieb SS, Meidna N, Yushak M. Prevention and reversal of nitrate tolerance in patients with congestive heart failure. N Engl J Med 1987; 317: 799-804.
6. Packer M. What causes tolerance to nitroglycerin? The 100 year old mystery continues. J Am Coll Cardiol 1990 19: 932-5.
7. Gori T, Parker JD. The puzzle of nitrate tolerance: pieces smaller than we thought? Circulation 2002; 106: 2404-8.
8. Gori T, Parker JD. Nitrate tolerance: a unifying hypothesis. Circulation 2002; 106: 2510-3.
9. Munzel T, Sayegh H, Freeman BA, Tarpey MM, Harrison DG. Evidence for enhanced vascular superoxide anion production in nitrate tolerance. a novel mechanism underlying tolerance and cross-tolerance. J Clin Invest 1995; 95: 187-94.
10. Munzel T, Daiber A, Muelsch A. Explaining the phenomenon of nitrate tolerance. Circ Res 2005; 97: 618-28.
11. Gupte SA, Okada T, Ochi R. Superoxide and nitroglycerin stimulate release of pgf2 alpha and txa2 in isolated rat heart. Am J Physiol 1996; 271: 2447-53.
12. Morcillo E, Redi PR, Dubin N, Ghodkaonkar R, Pitt B. Myocardial prostaglandin E release by nitroglycerin and modification by indomethacin. Am J Cardiol 1980: 45: 53-7.
13. Levine RI, Weksler BB, Tack-Goldanan K. Nitroglycerin stimulates synthesis of prostacyclin by cultured human endothelial cells. J Clin Invest 1981; 67: 762-9.
14. Waldman SA, Murad F. Cyclic GMP synthesis and function. Pharmacol Rev 1987; 39: 163-205.
15. Murad F. Cyclic guanosine monophosphate as mediator of vasodilation. J Clin Invest 1986; 78: 1-5.
16. Panzenbeck MJ, Balz A, Kaley G. Nitroglycerin and nitroprusside increase coronary blood flow in dogs by a mechanism independent of prostaglandin release. Am J Cardiol 1984; 53: 936-40.
17. Munzel T, Stewart DJ, Holtz J, Bassenge E. Preferential venoconstriction by cyclooxygenase inhibition in vivo without attenuation of nitroglycerin venodilation. Circulation 1988; 78: 407-15.
18. Ignarro LJ, Lippton H, Edwards JC, et al. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of s-nitrosothiols as active intermediates. J Pharmacol Exp Ther 1981; 218: 739-49.
19. Kukuvetz WR, Holzman A, Wurm A, Poch G. Evidence for cyclic GMP-mediated relaxant effects of nitro-compounds in coronary smooth muscle. Naunyn Schmiedeberg's Arch Pharmacol 1979; 310: 129-38.
20. Kowaluk EA, Fung HL. Dissociation of nitrovasodilator-induced relaxation from cyclic GMP levels during in vitro nitrate tolerance. Eur J Pharmacol 1990; 176: 91-5.
21. Matlib MA, Dube GP, Millard RW, et al. Studies on the mode of action of isosorbide dinitrate: a physiologic and biochemical approach. Am Heart J 1985; 110: 204-12.
22. Strein K, Bossert F, Bartsch W, Hooper R. Pharmacodynamics of organic nitrates. In vitro and in vivo studies. In: DG Julian, R Rittinghausen eds. Mononitrates II. Berlin: Springer-Verlag, 1987; 5-12.

23. Murad F, Rapoport RM, Fiscus R. Role of cyclic GMP in relaxation of vascular smooth muscle. J Cardiovasc Pharmacol 1985; 7 (suppl 3): S111-18.
24. Broadley KJ. The langendorff heart preparation. Reappraisal of its role as a research and teaching model for coronary vasoactive drugs. J Pharmacol Meth 1979; 2: 143-52.
25. Fallen EL, Elliot WC, Gorlin R. Apparatus for study of ventricular function and metabolism in the isolated perfused rat heart. J Appl Physiol 1967; 22: 836-9.
26. van Rossum JM. Cumulative dose-response curves. ii. Techniques for the making of dose-response curves in isolated organs and the evaluation of drugs parameters. Arch Int Pharmacodyn Ther 1963; 143: 299-330.
27. Raddino R, Poli E, Ferrari R, Visioli O. Effects of calcium entry blockers not connected with calcium channels inhibition. Gen Pharmacol 1987; 18: 431-6.
28. Ahlner J, Ljusegren ME, Grundstrom N, Axelsson KL. Role of oxide and cyclic GMP as mediators of endothelium-independent neurogenic relaxation in bovine mesenteric artery. Circ Res 1991; 68: 756-62.
29. Rapoport RM, Waldman SA, Ginsburg R, Molina CR, Murad F. Effects of glyceryl trinitrate on endothelium-dependent and independent relaxation and cyclic GMP levels in rat aorta and human coronary artery. J Cardiovasc Pharmacol 1987; 10: 82-91.
30. Raddino R, Pela G, Manca C, et al. Mechanism of action of human calcitonin gene-related peptide in rabbit heart and in human mammary arteries. J Cardiovasc Pharmacol 1997; 29: 463-70.
31. Ratz JD, McGuire JJ, Bennett BM. Enantioselective inhibition of the biotransformation and pharmacological actions of isoidide dinitrate by diphenyleneiodonium sulphate. Br J Pharmacol 1999; 126: 61-8.
32. The task force on Acute Hearth Failure of the European Society of Cardiology. Guidelines on the diagnosis and treatment of acute heart failure. Eur Heart J 2005; 26: 384-416.
33. The task force on Management of Stable Angina Pectoris of the European Society of Cardiology. Guidelines on the management of stable angina pectoris: executive summary: the task force on the management of stable angina pectoris of the European Society of Cardiology. Eur Heart J 2006; 27: 1341-81.
34. Macho P, Vatner SF. Effects of nitroglycerin and nitroprusside on large and small coronary vessel in conscious dogs. Circulation 1981; 64: 1101-7.
35. Bassenge E, Pohl E, Holtz J. Effect of different vasodilators on epicardial coronary arteries. In Cohn JN, Rittinghausen R eds. Mononitrates. Berlin: Springer-Verlag, 1985; 17-22.
36. Cauin C, Loutzenhiser R, van Breemen C. Mechanism of calcium antagonist-induced vasodilation. Annu Rev Toxicol 1983; 33: 373-96.