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Nitric oxide generation, tachyphylaxis and cross-tachyphylaxis from nitrovasodilators in vivo

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Abstract

Nitric oxide (NO) increments in exhaled air and changes in mean arterial pressure of anaesthetised rabbits were measured in order to study the NO generation from NO donors and tachyphylaxis in NO formation from nitroglycerin. Continuous infusions of isosorbide dinitrate, isosorbide-5-mononitrate and 3-morpholino-sydnonimine (SIN-1) evoked dose-dependent increases in exhaled NO, paralleled by decrements in mean arterial pressure. Repeated infusions of nitroglycerin resulted in attenuation ($P < 0.01$) of the NO increase from a given dose. Concurrent infusions of isosorbide dinitrate, isosorbide-5-mononitrate or nitroglycerin reduced the amount of NO emanating from the bioconversion of a given dose nitroglycerin as measured in the expired air ($P < 0.01$ for all drugs), indicating cross-tachyphylaxis. SIN-1 did not exhibit such cross-tachyphylaxis. In conclusion, measurements of exhaled NO can be a useful tool for exploration of nitrovasodilator tachyphylaxis. Cross-tachyphylaxis is only shared between some nitrovasodilators and is possibly not due to feedback from the generated NO.

Keywords: Organic nitrate; Vasodilator agent; Drug tolerance; Tachyphylaxis; Nitric oxide (NO)

1. Introduction

Nitroglycerin and chemically related compounds referred to as nitrovasodilators or NO-donors are commonly used in treatment for various diseases where temporary perfusion insufficiencies occur. A common mode of action for all nitrovasodilators is liberation of nitric oxide (NO) (Katsuki et al., 1977; Feelisch and Kelm, 1991; Noack and Feelisch, 1991). NO stimulates the formation of cyclic guanosine monophosphate (cGMP) in smooth muscles (Katsuki et al., 1977; Feelisch and Noack, 1987), thereby causing them to relax (Axelsson et al., 1979). In addition, involvement of cGMP-*independent* mechanisms mediating smooth muscle relaxation to endogenous NO (Bolotina et al., 1994) and the NO-donor 3-morpholino-sydnonimine (SIN-1) (Plane et al., 1996) have been suggested.

During administration of nitrates in high doses, in vivo or in vitro, there is a rapid attenuation of the hemodynamic or vasorelaxant effects, tachyphylaxis (Boegart and De Schaepdryver, 1968; Newman et al., 1990; Persson et al., 1994). This tachyphylaxis has been subjected to extensive investigation, since it has been hypothesised that the mechanisms involved also could be implicated to explain the nitrovasodilator tolerance seen in clinical praxis. Nitrovasodilator tolerance occurs during prolonged or frequent administration of nitrovasodilators in lower doses and leads to attenuation of their hemodynamic and anti-ischemic actions. However, the mechanisms underlying the nitrovasodilator tolerance in the clinical situation are likely multifactorial and the relative importance of the various mechanisms, including those responsible for the more rapid tachyphylaxis, remain unclear (see Fung and Bauer, 1994). However, reduced amounts of biologically active NO following reduced bioconversion of nitrovasodilators (Needleman and Johnson, 1973; Feelisch and Noack, 1987) and/or enhanced breakdown of the NO generated (see Münzel and Harrison, 1997) is likely to be one of the primary mechanisms responsible for both tachyphylaxis and nitrovasodilator tolerance. Several in vitro studies favour reduced bioconversion of nitrates as the major

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cause, at least regarding tachyphylaxis (Berkenboom et al., 1988; Mülsch et al., 1988; Forster et al., 1991; Chung and Fung, 1993).

However, there is controversy if the reduced bioconversion also is important for the nitrovasodilator tolerance in vivo, since studies of NO generation from nitroglycerin have shown enhanced NO production during long term treatment (Laursen et al., 1996). There is also strong evidence that prolonged treatment of rabbits with nitroglycerin increases endogenous rates of O_2^- production which in turn inactivates NO released from nitroglycerin (see Münzel and Harrison, 1997). Therefore, the current view of nitrate tolerance in vivo is that enhanced breakdown of NO rather than decreased NO generation causes tolerance.

We have demonstrated that NO can be measured in exhaled air (Gustafsson et al., 1991) and established a model in which we directly can study the kinetics of the bioconversion of nitroglycerin (Persson et al., 1994) and organic nitrates/nitrites (Cederqvist et al., 1994) to NO in vivo. By measuring the increase in airway concentrations of NO during continuous infusion of nitrovasodilators, we can get a picture of the net amount of formation and inactivation of NO generated from nitrovasodilators. In this model, increments in exhaled NO correlates to concomitant decreases in mean systemic arterial pressure. During nitroglycerin infusion, we found that NO in exhaled air increased and reached a peak value and then rapidly declined to a more stable ''plateau'', a pattern of rapid tachyphylaxis. Changes in thiol availability, NO synthase activity or pulmonary haemodynamics did not explain tachyphylaxis in this model (Persson et al., 1994).

The aim of the present study was to further characterise the tachyphylaxis during nitroglycerin infusions as measured by exhaled NO. Furthermore, we wanted to expand our model to other NO-donors and then investigate how these influenced the tachyphylaxis in NO generation from nitroglycerin.

2. Methods

2.1. Surgical procedures

New Zealand white rabbits of either sex and of body weight between 2 and 3 kg were anesthetized with pentobarbital sodium with an induction dose of 60 mg kg^{-1} given during 30 min via an ear vein. Prior approval was obtained from the local animal ethics committee.

Animals were placed in a supine position on the operating table, tracheotomized and ventilated by means of a Harvard Apparatus, model 683, rodent ventilator (South Natick, MA, USA). The respirator was supplied with NO-free air (ambient air filtered through a charcoal filter, 150×12 cm). Ventilation rate was 40 min^{-1} and the tidal volume adjusted to keep the end tidal CO_2 at 4.0–5.0% as measured by a ventilatory monitor (Oscar-Oxy, Datex, Helsinki, Finland). A continuous infusion containing glucose (2.75 g 100 ml^{-1}), dextran 70 (2.8 g 100 ml^{-1}), $NaHCO_3$ (0.7 g 100 ml^{-1}), and pentobarbital sodium (480 mg 100 ml^{-1}) was administered at a rate of 5 ml kg^{-1} h^{-1} by means of a STC-521 syringe pump (Terumo, Tokyo, Japan). A heparinized catheter was inserted in the left carotid artery for blood pressure recordings (pressure transducer P23 AC, Statham Instruments, Hato Rey, Puerto Rico) and sampling of blood gases. Another catheter was inserted in the right jugular vein for administration of drugs. The animals were paralyzed with pancuronium bromide (initial dose 1 mg kg^{-1} and 0.5 mg kg^{-1} h^{-1} given as bolus infusions. Body temperature was maintained at 38°C by means of a heating pad connected to a thermostat (Wittman-Hereaus, Heidelberg, Germany).

2.2. NO measurements

NO was analyzed in exhaled air as previously described (Persson et al., 1994) using a system from Aerocrine (Danderyd, Sweden) set at an integration time of 0.12 s, response time was 0.35 s and detection limit 1 ppb. NO in mixed exhaled air was sampled from the respirator outlet via a T-connection, sampling flow 40 ml min^{-1}. The other line from the respirator outlet T-connection was led into a beaker with water in such a way that the remainder of the expired air had to pass through the water creating a positive end-expiratory pressure set at 1–2 cm H_2O.

2.3. Experimental protocol

After completion of the operating procedures, a blood gas sample was taken in order to monitor the ventilator settings and acid–base status. The blood gas samples were analysed in a Radiometer ABL 300 acid–base laboratory blood gas analyser (Radiometer, Copenhagen, Denmark). The ventilator settings were adjusted and $NaHCO_3$ (0.6 M) buffer infusion added to maintain arterial blood gases at: pH 7.35–7.45, pCO_2 4.0–5.0 kPa, $pO_2 > 10$ kPa and base excess 0 to -5 mmol l^{-1}. Hereafter, the animals were allowed to stabilise for at least 20 min. Three different types of experiments were performed: (1) Dose–response curves for isosorbide dinitrate, isosorbide-5-mononitrate and SIN-1; (2) Experiments to study tachyphylaxis during repeated nitroglycerin infusions; (3) Experiments to study ''cross-tachyphylaxis'' by comparing the exhaled NO generation from nitroglycerin per se with the generation during concomitant infusion of isosorbide dinitrate, isosorbide-5-mononitrate, SIN-1 or nitroglycerin itself.

The NO-donors were given in a concurrent infusion of saline (100 μl kg^{-1} min^{-1}) functioning as carrier solution. The carrier solution was given by means of a syringe pump (Univentor 864, AgnTho's, Lidingö, Sweden).

2.4. Dose–response curves

Dose–response curves for isosorbide dinitrate, isosorbide-5-mononitrate and SIN-1 were constructed using three different groups of animals ($n = 4–7$), each group receiving only one of the NO-donors. The NO-donors were given as continuous infusions by means of a micro-infusion pump (CMA 100, Carnegie Medical, Stockholm, Sweden) during 10-min periods. NO increase in expired air was measured and the NO value just before start of the infusion was taken as control for the endogenous NO production. Between each dose, the animal was allowed to stabilise in arterial pressure and exhaled NO. The time needed for this varied between different NO-donors and dosages, however, the shortest period between two different doses was 20 min even if stable conditions were achieved prior to this. The NO-donors were given in a non-randomised increasing dose fashion.

After each dose of NO-donor acid–base status was corrected if needed, by means of buffer infusions ($NaHCO_3$ 0.6 M). This was necessary especially after NO-donor doses that caused a profound and long-lasting decrease in blood pressure (e.g., with SIN-1).

The changes of exhaled NO and mean arterial pressure from each measurement during the infusions was plotted and a correlation coefficient calculated in order to estimate the correlation between the two parameters.

2.5. Repeated infusions of nitroglycerin

In order to study the tachyphylaxis during repeated nitroglycerin infusions, one group of animals ($n = 6$) was given three 10 min infusions of nitroglycerin (0.1 mg kg^{-1} min^{-1}) with a 50-min interval between each infusion. Peak (maximal increase during infusion) and plateau (increase at the time when the infusion was stopped) values for NO increase in expired air were measured. The NO value just before start of the nitroglycerin infusion was taken as control for the endogenous NO production.

2.6. NO generation from GTN during concomitant infusion of nitrovasodilators

For investigation of potential cross-tachyphylaxis between nitroglycerin and the other NO donors tested, we studied how the different NO-donors (including nitroglycerin itself) were interacting with the NO generation from the third nitroglycerin infusion. Thus, similar to the above experiment, nitroglycerin (0.1 mg kg^{-1} min^{-1} for 10 min) was infused at 50-min intervals (*indicator infusions*). However, 45 min prior to the third nitroglycerin infusion, an infusion of either isosorbide dinitrate (0.1 mg kg^{-1} min^{-1}, $n = 6$), isosorbide-5-mononitrate (0.2 mg kg^{-1} min^{-1} $n = 5$), SIN-1 (0.1 mg kg^{-1} min^{-1}, $n = 6$) or nitroglycerin (0.03 mg kg^{-1} min^{-1}, $n = 4$) was started and continued throughout the experiment (*trial infusion*). Each animal received only one *trial infusion* of NO-donor. The NO increase in exhaled air from the *trial* infusion per se was measured just before the start of the nitroglycerin *indicator infusion*. This was done to analyse if there was a correlation between the amount of NO generated from the *trial infusion* and the amount of cross-tachyphylaxis induced. The group given only repeated nitroglycerin *indicator infusions* described above was used as control.

The reason for studying the NO generation from the third nitroglycerin infusion was that the tachyphylaxis for nitroglycerin per se (measured as the difference in NO generation between one nitroglycerin infusion compared to the NO generation from the previous infusions) was minute for the third infusion as compared to the second infusions (see Section 3).

2.7. Statistics

Statistical data are given as mean and standard error of the mean (S.E.M.). Statistical significance was calculated by means of Student's *t*-test after test for normal distribution. Pearson's Product Movement Correlation was used to calculate correlations between changes in exhaled NO and changes in mean arterial pressure. For calculation of statistical difference between repeated measurements, One-way ANOVA and Tukey post hoc analysis were used. All statistical calculations were done by using a computer program (SigmaStat, Jandel, San Rafael, CA, USA).

2.8. Drugs

L-NAME (N^G-nitro-L-arginine methyl ester) and isosorbide dinitrate were purchased from Sigma, St Louis, Missouri, USA, SIN-1 was from Cassella, Frankfurt/Main, Germany, heparin from Kabi Vitrum, Stockholm, Sweden, pancuronium bromide (Pavulon®) from Organon, Oss, Holland, pentobarbital from Apoteksbolaget, Stockholm, Sweden, dextran 70 (Macrodex®) from Pharmacia Infusion, Uppsala, Sweden, nitroglycerin (Perlinganit®) and isosorbide-5-mononitrate were from Schwartz Pharma, Monheim, Germany.

3. Results

3.1. Baseline conditions

Mean arterial pressure and NO concentrations in exhaled air remained at 84 ± 2 cm H_2O and 16.0 ± 0.4 parts per billion (ppb) during the 20 min control period ($n = 50$).

3.2. Dose–response profiles of exhaled NO

All NO-donors tested caused dose-dependent increments in exhaled NO and concurrent dose-dependent decrements in blood pressure. However, as shown in

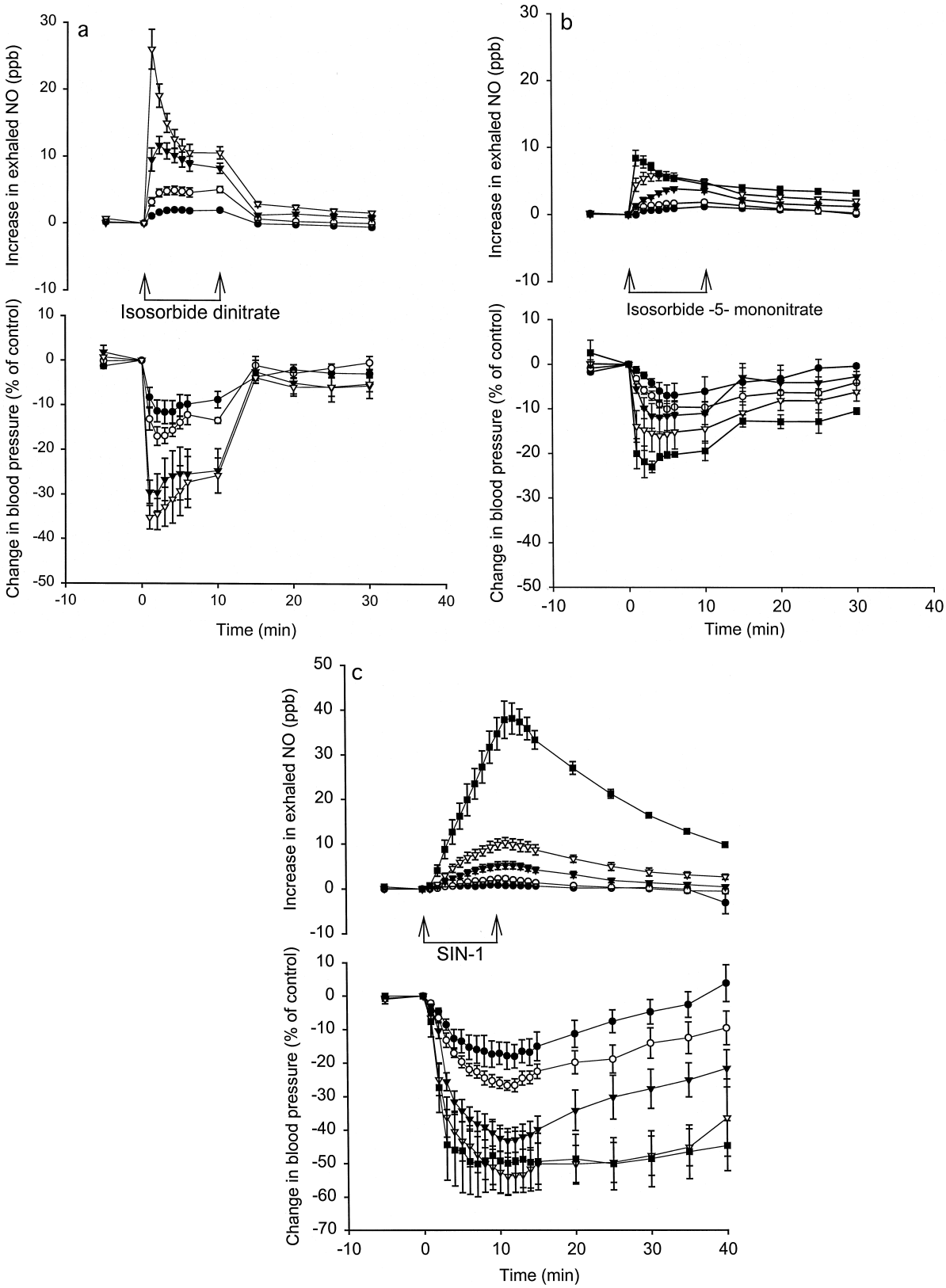

Fig. 1. Artificially ventilated pentobarbital anesthetized rabbits. Time course of increments of NO in mixed exhaled air (upper panels) and decrements in mean arterial blood pressure (lower panels) in response to continuous infusions of different NO-donors (start and stop of infusions marked with arrows). (a) Isosorbide dinitrate 0.01 (●), 0.03 (○), 0.1 (▼) and 0.3 (▽) mg kg^{-1} min^{-1}, $n = 6$. (b) Isosorbide-5-mononitrate 0.1 (●), 0.3 (○), 1.0 (▼), 3.0 (▽) and 10.0 (■) mg kg^{-1} min^{-1}, $n = 4–7$. (c) SIN-1 0.01 (●), 0.03 (○), 0.1 (▼), 0.3 (▽) and 1.0 (■) mg kg^{-1} min^{-1}, $n = 3–4$. All values given as means ± S.E.M.

Fig. 1, the patterns of the NO changes in exhaled air were substantially different between the compounds.

In the highest dose of isosorbide dinitrate tested (0.3 mg kg^{-1} min^{-1}), there was an initial peak (within 1 min.) in exhaled NO after which NO decreased to and stabilized at values closer to control. This ''peak and plateau'' appearance was less pronounced or absent at lower doses of isosorbide dinitrate (Fig. 1).

SIN-1 infusions caused a continuous increase in exhaled NO throughout the duration of the infusion and NO in exhaled air reached its maximal value at the end of the infusion or after the infusion was stopped. At the highest dose tested (1.0 mg kg^{-1} min^{-1} for 10 min), NO peaked between 11 and 12 min and was still significantly ($P < 0.05$) increased above basal levels 60 min after starting the infusion (data not shown).

Isosorbide-5-mononitrate caused a continuous increase in NO which reached its maximal value within the time of the infusions and then decreased again. The magnitudes of the NO increments and the decrements in mean arterial pressure were substantially lower with isosorbide-5-mononitrate as compared to isosorbide dinitrate. There were also marked differences between isosorbide dinitrate and isosorbide-5-mononitrate in how long time the NO increments and blood pressure changes lasted after the infusions were stopped (Fig. 1).

The half-life ($T_{1/2}$) for the increments of NO upon termination of the infusions was: isosorbide dinitrate 90 ± 10 s, isosorbide-5-mononitrate 147 ± 30 s and SIN-1 756 ± 61 s. There was a significant difference ($P < 0.01$) between $T_{1/2}$ for SIN-1 and $T_{1/2}$ for the other NO-donors tested.

Correlation curves for changes in exhaled NO and mean arterial pressure revealed almost linear correlation for NO increase ≤ 10 ppb. However, for NO ≥ 10 ppb, further increase in NO only weakly correlated to decrease in mean arterial pressure (data not shown). Therefore, correlation coefficients between exhaled NO and decrease in mean arterial pressure were calculated for isosorbide dinitrate, isosorbide-5-mononitrate and SIN-1 for NO increments ≤ 10 ppb using the data for mean arterial pressure and NO obtained from the dose–response experiments (Table 1). The same correlation regarding nitroglycerin was obtained by using the data from the repeated nitroglycerin infusions. The correlation coefficient for change in NO vs. mean arterial pressure during SIN-1 was significantly ($P < 0.001$) lower than compared to the other studied nitrovasodilators. However, the difference in correlation coefficients between nitroglycerin, isosorbide dinitrate and isosorbide-5-mononitrate did not reach significance.

Table 1
Correlation between changes in exhaled NO and mean arterial pressure during NO-donor infusions in anesthetized rabbits
Measurements were obtained during continuous infusions of NO-donors. Restricting calculations to conditions where NO increased ≤ 10 ppb, correlations to the corresponding decrease in mean arterial pressure were determined. For further explanation see text. Correlation coefficients given as means ± S.E.M.

Drug	Correlation coefficient	*R* (mean)	Range	Number of animals
Isosorbide dinitrate	−2.390 ± 0.345	0.852	0.806–0.909	6
Isosorbide-5-mononitrate	−1.879 ± 0.955	0.610	0.022–0.937	7
SIN-1	−5.450 ± 0.247	0.777	0.669–0.859	4
Nitroglycerin	−2.634 ± 0.349	0.818	0.664–0.914	5

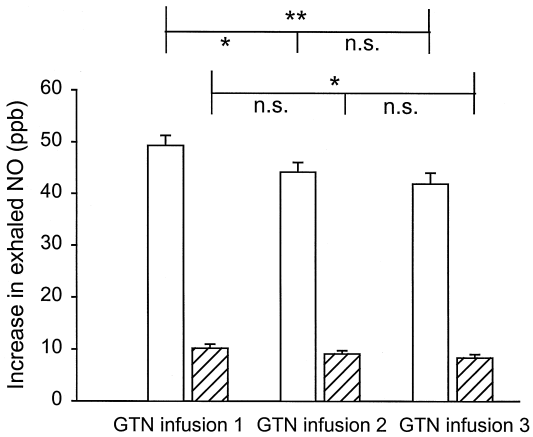

Fig. 2. Artificially ventilated pentobarbitone-anesthetized rabbits. Effects of repeated infusions of nitroglycerin, 0.1 mg kg^{-1} min^{-1} for 10 min with 50-min intervals, on NO concentration in mixed exhaled air. NO was measured as increase in NO concentration as compared to control immediately before infusion was started. Open columns represent the maximal or ''peak'' increase in NO during the infusions. Hatched columns represent ''plateau'' NO values achieved immediately before the infusions were stopped. * indicates $P < 0.05$ and ** indicates $P < 0.01$ in comparisons between values, n.s indicates non-significance in comparisons. All values given as means ± S.E.M.

3.3. Repeated infusions of nitroglycerin

Three repeated infusions of nitroglycerin (0.1 mg kg^{-1} min^{-1} for 10 min) at 50-min intervals were applied. The resulting peak and plateau values of NO, in exhaled air, are given in Fig. 2. The peak increase in exhaled NO concentration significantly decreased between the first and second infusion but stabilised during the second and third infusions where no significant difference was observed. The plateau phase of the third infusion was significantly reduced as compared with the first, whereas there was no significant difference in the plateau phase between the first and second or second and third infusion.

The decrease in the peak concentration of NO in exhaled air between the infusions was accompanied by sig-

Table 2
Increase in exhaled NO from *trial* infusion just before start of *indicator* infusion
Artificially ventilated pentobarbitone-anesthetized NZW rabbits: The effect per se on NO concentration (ppb) in mixed exhaled air by the ''trial'' infusions of NO-donors given in cross-tolerance experiments. The increase in NO concentration was determined at 45 min of respective *trial* infusion, i.e., just before the start of the *indicator* infusion. All values given as means ± S.E.M.

NO-donor, *trial* infusion	Dose (mg kg^{-1} min^{-1})	Dose, NO-donating groups (μmol kg^{-1} min^{-1})	NO-increase at 45 min by *trial* infusion (ppb)
Nitroglycerin	0.03	0.396	3.3 ± 0.70
SIN-1	0.1	0.480	6.7 ± 0.65
Isosorbide dinitrate	0.1	0.850	6.1 ± 0.50
Isosorbide-5-mononitrate	0.2	1.040	1.7 ± 0.35

nificant ($P < 0.05$) attenuation of the peak decrease in blood pressure during the third ($-28 \pm 3\%$) as compared to the first ($-38 \pm 4\%$) nitroglycerin infusion ($n = 6$). Yet no statistically significant difference in the blood pressure decrease during the plateau phase between the three infusions was observed.

3.4. NO generation from nitroglycerin during concomitant infusion of nitrovasodilators

The effects of the *trial infusions* of nitrovasodilators per se on exhaled NO are given in Table 2.

Trial infusion with, isosorbide dinitrate, isosorbide-5-mononitrate or nitroglycerin caused significantly reduced peak as well as plateau concentrations in exhaled NO from the *indicator* nitroglycerin *infusion* (Fig. 3a,b,d).

Trial infusion with SIN-1 had no significant effect on the peak but significantly ($P = 0.033$) increased the plateau concentration in exhaled NO from the *indicator* nitroglycerin infusion (Fig. 3c).

The reduction in the *indicator* peak and plateau NO was significantly stronger ($P < 0.001$ for each) with the concurrent infusion of isosorbide dinitrate as compared to the isosorbide-5-mononitrate infusion.

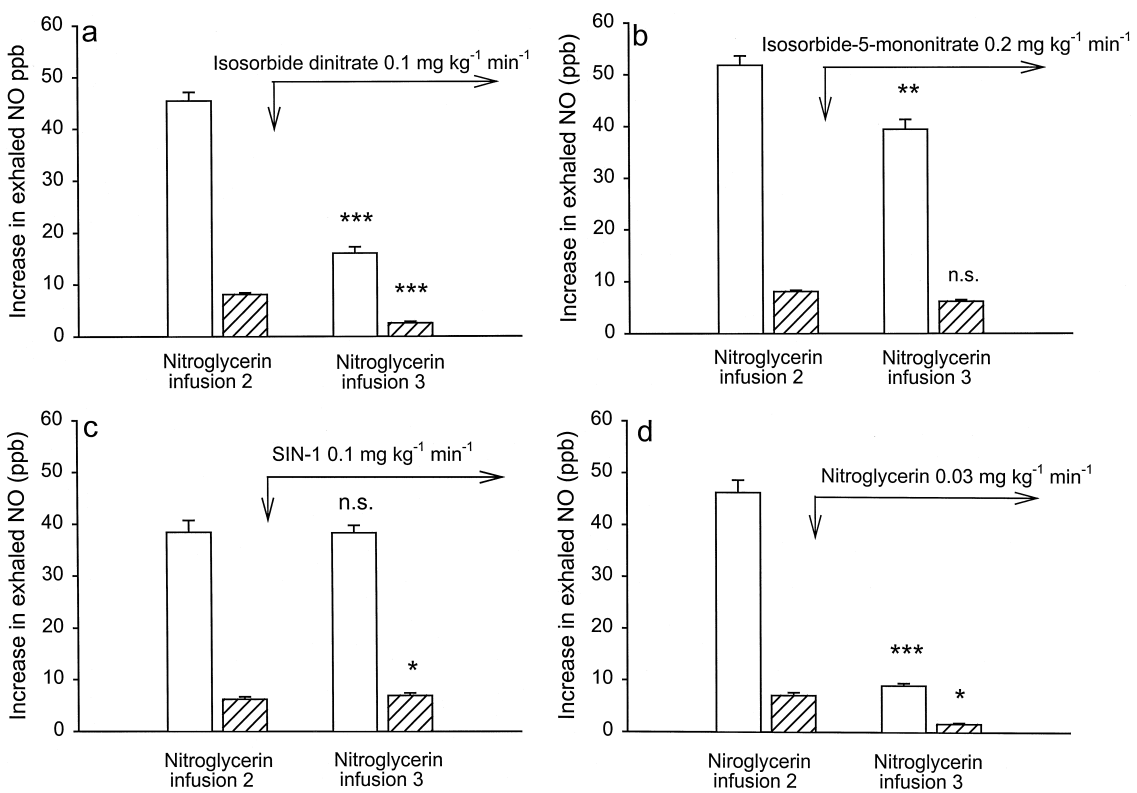

Fig. 3. Artificially ventilated pentobarbitone-anesthetized rabbits: Increase in exhaled NO from repeated doses of nitroglycerin (0.1 mg kg^{-1} min^{-1} for 10 min at 50-min intervals). The second nitroglycerin infusion (nitroglycerin infusion 2) was given without, and the third nitroglycerin infusion (nitroglycerin infusion 3) with concurrent infusion of other NO-donors. (a) Concurrent infusion of isosorbide dinitrate, (b) concurrent infusion of isosorbide-5-mononitrate, (c) concurrent infusion of SIN-1, (d) concurrent infusion of nitroglycerin. Open columns represent the maximal or ''peak'' increase in NO during the nitroglycerin indicator infusions. Hatched columns represent ''plateau'' NO values achieved just before the infusions were stopped. * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, in comparison to a control infusion 3 in absence of concurrent infusion of NO-donor, n.s indicates lack of significance in comparison. All values given as means ± S.E.M.

4. Discussion

The present study demonstrates that NO is formed from nitroglycerin, SIN-1, isosorbide dinitrate and isosorbide-5-mononitrate in vivo and can be measured in exhaled air from rabbits. The nitrovasodilators exhibit individual NO-donating capacities and patterns of NO-release. The increase in NO is paralleled by a fall in mean arterial pressure. Most importantly, we demonstrate a tachyphylaxis in the levels of active NO emanating from nitroglycerin as measured in exhaled air. This tachyphylaxis is present both upon repeated nitroglycerin infusions and as cross-tachyphylaxis during concomitant infusions of isosorbide dinitrate or isosorbide-5-mononitrate while SIN-1 does not have this effect.

The present data on nitroglycerin and isosorbide dinitrate confirm the peak and plateau appearance observed in our previous study (Persson et al., 1994). In these experiments, inhibition of the endogenous NO-synthesis did not affect the generation of NO from nitroglycerin suggesting that the increased NO detected in exhaled air was exclusively derived from nitroglycerin and that endogenous NO was not involved in the development of the tachyphylaxis observed. Furthermore, the peak and plateau pattern did not change with altered pulmonary vascular resistance (Persson et al., 1994), i.e., it was not due to altered distribution within the lung (i.e., changes in pulmonary circulation resulting in altered partition of NO into different lung compartments). Further strengthening this is that the peak plateau pattern of exhaled NO remains even with nitroglycerin administration to buffer perfused lungs (Agvald et al., unpublished observations). The peak plateau patterns observed in this and our previous study are well in line with similar variations over time of cGMP levels in lung, heart and aorta after bolus injection of nitroglycerin (Haj-Yehia and Benet, 1994). The similarity in cGMP patterns between lung and aortic tissue in their study also supports the notion that NO measured in expired air occurs in parallel with the NO generated in the circulatory system and hence, that our model is relevant for studying the mechanisms involved in the bioconversion of nitrovasodilators to NO. The peak and plateau appearance for NO in exhaled air upon a bolus injection of nitroglycerin has also been observed in humans (Marczin et al., 1997) confirming the relevance of this rabbit model.

The model applied in this study has the benefit of being an in vivo system with on-line high-resolution detection of the NO generated (the temporal resolution is sufficiently high to detect with high accuracy changes in the NO concentration within seconds). In contrast to other indirect ''in vivo'' methods, i.e., spin trapping which measures the integrated production over considerable longer time (Mülsch et al, 1995b), the rapid response time enables detection of the peak and plateau pattern. NO produced in the body is continuously degraded partly by oxygen-derived radical metabolites such as superoxide ions (see Freeman, 1994). The NO measured in the exhaled air is the net amount of formation and inactivation. Thus, NO measured in exhaled air gives a picture of the NO that actually reaches the cells, that is, is active in vivo. One disadvantage with the technique is, on the other hand, that it is difficult to tell if changes in NO are due to changed production or elimination of the substance. Accordingly, tachyphylaxis in NO exhalation may be due to several mechanisms, one of which is reduced NO formation in the lung. The spin trap technique on the other hand is largely insensitive to NO elimination and therefore measures NO formation rates in tissues.

Homogenates from rabbit lung have been shown to perform bioconversion of both nitroglycerin and isosorbide dinitrate to NO (Mülsch et al., 1995a). However, the type of cells that are responsible for the generation of the NO measured in exhaled air remains to be elucidated. Among cells found in the lung, several have been shown to be able to perform the bioconversion of nitrovasodilators to NO, e.g., endothelial and vascular smooth muscle cells (Feelisch and Kelm, 1991) and platelets (Weber et al., 1996).

There was a conspicuous difference in the profiles of NO change in exhaled air between the drugs studied. The peak and plateau appearance which was observed at the higher doses of both nitroglycerin and isosorbide dinitrate could reflect either reduced bioconver-sion of the drugs or enhanced elimination of the NO donated during the infusion. At least two enzyme systems capable of producing NO from nitroglycerin and isosorbide dinitrate have been described (Servent et al., 1989; Yeates et al., 1989). However, there is still controversy about their role in bioconversion of nitroglycerin in the vascular system (Braun et al., 1995). In addition, there are also possible non-enzymatic degradation pathways since studies on cell free preparations have shown that thiol-containing substances, such as L-cysteine, directly interact with nitroglycerin resulting in NO or in nitrosothiol formation (Ignarro et al., 1981; Feelisch and Noack, 1987). However, the non-enzymatic degradation does not seem to play a crucial role in vivo since protein denaturation of tissue homogenates or purified cells virtually eliminates NO formation (Feelisch and Kelm, 1991; Kurz et al 1993).

In spite of high doses, isosorbide-5-mononitrate gave moderate effects on both NO and reductions in mean arterial pressure compared to the other NO-donors tested. This was likely because the dose-regimen used (i.e., non-randomised increasing dose fashion) caused tachyphylaxis between the infusions that was more pronounced for isosorbide-5-mononitrate compared to the other drugs tested.

In contrast to the other drugs tested, SIN-1 caused NO to increase even after cessation of the infusion and the $T_{1/2}$ for NO generation and blood pressure effect was considerably longer compared to the other nitrovasodilators tested. This might be due to that SIN-1 has to convert to SIN-1A (halftime 5 min), which then decomposes in an oxygen

consuming reaction into NO. The halftime for this reaction is about 30 min (Böhme et al., 1982; Feelisch et al., 1989).

The dose–response curves for blood pressure were a reflection of the NO generated from the nitrovasodilators. However, there was no complete correlation between NO in exhaled air generated from the nitrovasodilators and blood pressure reductions over the full dose-range investigated in this study. At about 60% decrease in mean arterial pressure, further increase in NO seemed to have little or no further effect on mean arterial pressure. The mechanism for this may include that the cGMP-system at this level of NO generation already is maximally activated or that physiological counter-regulatory mechanisms come into play, preventing further blood pressure decrements although NO generation is further increased at the higher doses of nitrovasodilators (see Fung and Bauer, 1994).

The linear correlation between NO increase ≤ 10 ppb in exhaled air and blood pressure decrease was significantly steeper for SIN-1 as compared to the other NO-donors tested. This could be due to different distribution patterns for the drugs, i.e., SIN-1 releasing a larger fraction of its NO in the systemic circulation at the level of resistance vessels than in the lung. Different distribution patterns could in turn be due to different lipophilicity of the drugs (Noack, 1984) or different distribution of the enzymes that mediate bioconversion of the various drugs to NO. There is also a possibility that SIN-1, aside from its capacity to induce vasodilation through the generation of NO has additional mechanisms of action independent of NO-generation such as direct activation of K-channels (Plane et al., 1996).

In order to investigate cross-tachyphylaxis between nitrovasodilators, we studied the effect of the respective nitrovasodilator, administered at a low dose for a comparatively long duration (*trial infusion*) on the response to short-term high-dose infusion of nitroglycerin (*indicator infusion*).

Our original aim with the cross-tachyphylaxis studies was actually to investigate how nitroglycerin and isorbide dinitrate interacted with each other. In order to get unmistakable effect, we chose a dose of isosorbide dinitrate that produced a marked effect on the blood pressure (plateau values about -25% from control). From the result from these original experiments the questions arose, is it the isosorbide dinitrate molecule itself, the NO generated or some other mechanism, e.g., isosorbide dinitrate metabolite, which cause the cross-tachyphylaxis? The metabolite of isosorbide dinitrate, isosorbide-5-mononitrate (Tam et al., 1988) was added in about twice the dose (to yield fairly the same number of nitrite groups) to evaluate if the molecular structure in itself was causing the tachyphylaxis (i.e., through inhibiting the active site in nitroglycerin metabolizing enzyme). This was clearly not the case since the isosorbide-5-mononitrate in spite the higher dose caused less cross-tachyphylaxis than did isosorbide dinitrate. To test whether the NO generated caused the tachyphylaxis, SIN-1 in a dose producing slightly higher increase in NO levels than the ISDN dose was given. However, SIN-1 lacked any cross-tachyphylaxis inducing effect towards nitroglycerin. Thus, the data favours mechanisms other than product inhibition of the bioconversion mechanism by NO, as accountable for the development of tachyphylaxis.

The much higher potency of ISDN to induce tachyphylaxis, compared to isosorbide-5-mononitrate pointed to a mechanism coupled to the bioconversion of these drugs to NO. To test this, a dose of nitroglycerin giving only half the amount of NO, compared to the isosorbide dinitrate trial dose was tested. However, this nitroglycerin trial dose exhibited more tachyphylaxis than isosorbide dinitrate, suggesting that the NO generated from isosorbide dinitrate is not responsible for cross-tachyphylaxis, and that other differences in their metabolism are responsible for difference in tachyphylaxis.

In this study, the observations of the generation of NO from nitrovasodilators confirm that NO is generated from nitrovasodilators in vivo. Repeated nitroglycerin infusions or concurrent infusions of isosorbide dinitrate, isosorbide-5-mononitrate or nitroglycerin reduce the NO generation from a given dose of nitroglycerin, that is, induce cross-tachyphylaxis with reduced capacity to generate NO. SIN-1 lacks any such cross-tachyphylaxis-inducing effect despite its ability to generate NO in vivo. In conclusion, exhaled NO measurement can be used for exploration of nitrovasodilator tachyphylaxis and tachyphylaxis development in a nitrovasodilator is not due to an effect per se by the NO generated. Thus, cross-tachyphylaxis in NO generation is only shared between some nitrovasodilators and is probably not due to feedback from the generated NO molecule itself.

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