Dependence of the metabolism of nitric oxide (NO) in healthy human whole blood on the oxygenation of its red cell haemoglobin

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Plasma or whole venous or arterialized blood from healthy human donors was incubated with NO (50–300 μM), and the resulting formation of methaemoglobin (MetHb), nitrosyl haemoglobin (HbNO), and plasma nitrite and nitrate were measured. In plasma, NO was converted to nitrite and nitrate in a ratio of 5:1. In arterial blood (O_2 sat. 94–99%) NO was almost quantitatively converted to nitrate and MetHb. No nitrite was detected and HbNO formation was low. In venous blood (O_2 sat. 36–85%) more HbNO and less nitrate was formed, in comparison to arterialized blood. We propose that NO liberated from endothelium of conductance and resistance vessels is taken up by red blood cells and inactivated by HbO_2 via stoichiometric conversion to MetHb and nitrate.

Keywords: Blood; EDRF; endothelium; erythrocytes; haemoglobin; methaemoglobin; nitrate; nitric oxide; nitrite

Introduction

NO has potent biological activities as a vasoactive, cytotoxic, platelet regulatory, and neurotransmitter agent (Furchgott & Zawadzki, 1980; see Moncada *et al.*, 1991 for references). Accumulating data also indicate a significant role for NO in several settings of cardiovascular disease (Gordon *et al.*, 1989; Drexler *et al.*, 1991). We assumed that quantitative methods to estimate NO formation might facilitate further evaluation of some of its physiological and pathophysiological roles. The development of such methods relies upon proper knowledge about the inactivation and elimination of NO from the intact organism. In this paper we describe a route by which NO in human blood is converted to nitrate, i.e. a metabolite that is readily eliminated via the kidneys.

Methods Portions of venous blood from healthy donors were incubated with NO (AGA Special Gas, final conc. 50–200 mM) with or without previous oxygenation. O_2 saturation was estimated with a standard method. The incubation was interrupted by separation of the blood into cells and plasma followed by freezing of the cell fraction at 70 K in electron paramagnetic resonance (EPR) tubes. Plasma was kept at -20°C until analysis. Other plasma fractions were separated before incubation with NO as above. The EPR spectra of the blood cell fraction were recorded for MetHb and HbNO at a microwave frequency of 9.22 GHz and a power of 20 mW from about 500 to 3500 gauss with a modulation amplitude of 20 gauss. Plasma levels of nitrite and nitrate were analyzed with liquid chromatography/uv detection at 214 nm after separation of proteins with ultrafiltration, and verified with gas chromatography/mass spectrometry (stable isotope dilution with $^{15}\text{NO}_3^-$, conversion to nitrotoluene, negative ion-chemical ionization, selective monitoring of m/e 136 for endogenous nitrate and m/e 137 for the ^{15}N -labelled internal standard).

Results Basal plasma nitrate was $44 \pm 3.8 \mu\text{M}$ (mean $\pm$ s.e., $n = 20$). Basal plasma nitrite was below $1 \mu\text{M}$; often no nitrite

could be detected. The basal levels of MetHb and HbNO were 19 ± 2.0 ($n = 20$) and 1.2 ± 0.3 ($n = 20$) units, respectively. Incubation of arterialized blood (O_2 sat. $96 \pm 0.8\%$) with NO for 2 min resulted in dose-dependent increases in the formation of nitrate and MetHb (Figure 1). At the highest NO concentration (200 μM) nitrate in plasma reached a level of 203 μM , suggesting almost quantitative conversion of NO to nitrate. In parallel, MetHb was elevated to about 140 units. HbNO increased very little, to about 11 units at 200 μM NO. Prolongation of incubation time to 15 min revealed mainly the same pattern: plasma nitrate was 237 μM , and MetHb and HbNO were 151 and 10 units, respectively (Figure 1). Incubation of venous blood (O_2 sat. $61 \pm 8.5\%$) with NO for 2 min revealed a different pattern. Nitrate and MetHb levels increased in parallel, but to lower concentrations (130 μM and 135 units, respectively) than when NO was incubated with arterialized blood (Figure 1). In contrast, the formation of HbNO was markedly enhanced, to 92 units at a NO concentration in the incubate of 200 μM (Figure 1). When the incubations of venous blood with NO were prolonged to 15 min, the products formed were more similar to those obtained in arterialized blood. Plasma nitrate and MetHb increased, to 260 μM and 260 units, respectively. HbNO was mainly unaffected after 15 min compared to after 2 min of incubation (Figure 1). Incubation of plasma with NO (200 μM , $n = 3$) for 15 min resulted in semiquantitative conversion to nitrite and nitrate, in a ratio of about 5:1. These levels of nitrite and nitrate were stable.

Discussion Plasma, in comparison to whole blood, was rather inefficient in converting NO to nitrite in the present experiments, highlighting the activity of the blood cell fraction in the inactivation of NO. Since the red cells are the most abundant, and the conversion of NO to nitrate was found to involve haemoglobin, it appears that the conversion occurred in the erythrocytes. Furthermore, the conversion of NO to nitrate was more rapid in blood with high compared to low oxygen saturation of the haemoglobin. This strongly suggests that HbO_2 acted as oxygen donor to the NO molecule in its conversion to nitrate. Conversion of NO to nitrate by oxygen also involves a one electron transfer to the resulting NO_3^- molecule. Our data indicate that the ferrous haeme of the haemoglobin acted as electron donor, to be converted to

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Figure 1 Dose-response curves demonstrating the formation of nitrate (■), methaemoglobin (○), and nitrosyl haemoglobin (△) in whole arterialised (O_2 saturation 94–99%) or venous (O_2 sat. 36–85%) blood incubated with nitric oxide (NO, 50–200 μM) is indicated on the horizontal axis) for 2 or 15 min. Symbols indicate mean of 4–6 observations with s.e. shown by vertical bars. Concentrations on vertical axis: nitrate in μM , methaemoglobin and nitrosyl haemoglobin in EPR units. One EPR unit corresponds to about 1 μM of methaemoglobin and 0.1 μM of nitrosyl haemoglobin.

ferric haeme, inasmuch as the amount of methaemoglobin formed increased in parallel to the formation of nitrate. These data consequently suggest that NO is converted to nitrate according to the formula

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