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Is carbon monoxide-mediated cyclic guanosine monophosphate production responsible for low blood pressure in neonatal respiratory distress syndrome?

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[1]*Department of Neonatology, Wilhelmina Children's Hospital, University Medical Center, Utrecht, and Departments of* [3]*Cardiology and* [4]*Obstetrics, Leiden University Medical Center, Leiden, The Netherlands; and* [2]*Department of Pediatrics, Stanford University School of Medicine, Stanford, California*

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van Bel, Frank, Vincent Latour, Hendrik J. Vreman, Ronald J. Wong, David K. Stevenson, Paul Steendijk, Johannes Egberts, and Tannette G. Krediet. Is carbon monoxide-mediated cyclic guanosine monophosphate production responsible for low blood pressure in neonatal respiratory distress syndrome? *J Appl Physiol* 98: 1044–1049, 2005. First published October 29, 2004; doi:10.1152/japplphysiol.00760.2004.—Infant respiratory distress syndrome (RDS) involves inflammatory processes, causing an increased expression of inducible heme oxygenase with subsequent production of carbon monoxide (CO). We hypothesized that increased production of CO during RDS might be responsible for increased plasma levels of vasodilatory cGMP and, consequently, low blood pressure observed in infants with RDS. Fifty-two infants (no-RDS, $n = 21$; RDS, $n = 31$), consecutively admitted to the neonatal intensive care unit (NICU) between January and October 2003 were included. Hemoglobin-bound carbon monoxide (COHb), plasma cGMP, plasma nitric oxide (NOx), and bilirubin were determined at 0–12, 48–72, and at 168 h postnatally, with simultaneous registration of arterial blood pressure. Infants with RDS had higher levels of cGMP and COHb compared with no-RDS infants (RDS vs. no-RDS: cGMP ranging from 76 to 101 vs. 58 to 82 nmol/l; COHb ranging from 1.2 to 1.4 vs. 0.9 to 1.0%). Highest values were reached at 48–72 h [RDS vs. no-RDS mean (SD): cGMP 100 (39) vs. 82 (25) nmol/l ($P < 0.001$); COHb 1.38 (0.46) vs. 0.91 (0.26)% ($P < 0.0001$)]. Arterial blood pressure was lower and more blood pressure support was needed in RDS infants at that point of time [RDS vs. no-RDS mean (SD): mean arterial blood pressure 33 (6) vs. 42 (5) mmHg ($P < 0.05$)]. NOx was not different between groups and did not vary with time. Multiple linear regression analysis showed a significant correlation between cGMP and COHb, suggesting a causal relationship. Mean arterial blood pressure appeared to be primarily correlated to cGMP levels ($P < 0.001$). We conclude that a CO-mediated increase in cGMP causes systemic vasodilation with a consequent lower blood pressure and increased need for blood pressure support in preterm infants with RDS.

preterm; guanosine 3′,5′-cyclic monophosphate; hypotension

A COMMON HEMODYNAMIC PROBLEM among prematurely born neonates is the occurrence of low blood pressure during the first days of life, especially in ventilated infants with neonatal respiratory distress syndrome (RDS) (11). The possible role of a hemodynamically significant patent ductus arteriosus (PDA) in low blood pressure is well understood. However, diminished peripheral vascular resistance appears to be another important factor (11). In a recent study of our group in premature infants with and without RDS, the severity of RDS was positively correlated with (increased) plasma levels of cGMP and with the need for antihypotensive therapy (12). The origin of the increased cGMP levels has remained unclear, because no evidence has been found for the upregulation of the L-arginine-nitric oxide (NO) production pathway, normally mediating many biological functions, including cGMP-induced vascular smooth muscle relaxation, and as such playing an active role in the regulation of arterial blood pressure (12, 17).

The increased plasma cGMP levels found in the infants with RDS are therefore most likely due to the activation of another cGMP-generating pathway in addition to the NO pathway. In this regard, the activation of cGMP by CO could play an important role. Under physiological conditions, CO is primarily produced by oxidative degradation of heme (22). However, CO production may be increased through increased expression of inducible heme oxygenase (HO-1) in situations of oxidative stress and inflammation (2) and nonenzymatic lipid peroxidation, as evaluated through measurements of malondialdehyde (MDA) (21). Increased levels of (exhaled) CO have been reported in patients with pulmonary diseases, such as asthma, and CO is considered under these conditions to be a marker of oxidative stress (6, 9, 23).

Because RDS has also been associated with inflammatory processes in the lung (1, 14), the aim of the present study was to investigate the relationship between plasma levels of vasodilatory cGMP and arterial blood pressure and the role of endogenous production of CO, as indexed by carboxyhemoglobin (COHb) in blood, in this relationship.

We hypothesize that, in infants with RDS, an increase in cGMP leads subsequently to lower arterial blood pressures and that CO has a mediating role here.

PATIENTS AND METHODS

The study was approved by the local ethics committee. After informed parental consent was received, all premature infants with a gestational age <32 wk, consecutively admitted to the neonatal intensive care unit between January and October 2003, were included in the study.

Obstetrical and neonatal data were collected prospectively.

At 0–12, 48–72, and 168 h of age, blood was sampled for determination of COHb, plasma total bilirubin, plasma cGMP, and plasma NO (NOx; see below). Simultaneously, mean arterial blood pressure (MABP) was registered, and treatment modalities for low

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blood pressure (volume expanders, cardioinotropics, corticosteroids) were recorded. Blood was sampled via an indwelling arterial catheter.

COHb determinations were made with a sensitive and accurate gas chromatographic method, and its concentrations were expressed as a percentage of total hemoglobin concentration, which was quantitated by the cyanmethemoglobin method, both as previously described (19, 20). With this method, the within-day and between-day coefficients of variation for reference blood samples are 3% and 8%, respectively (19). Plasma cGMP was determined using a commercially available enzyme immunoassay (Amersham International, Little Shelfort, Buckinghamshire, UK) and expressed as nanomoles per liter. Plasma NOx was assessed by measuring NO_2^- in plasma samples after reduction of NO_3^- to NO_2^- with spongy cadmium and measurement with the Griess reagent at 540 nm (5).

Arterial blood pressure was measured continuously from the arterial catheter (placed into the umbical, radial, or posterial tibial artery) with a pressure transducer to the blood pressure module of the monitor (Hewlett Packard Viridia Component Monitoring System, version A.0). To assess the intensity of blood pressure support, a "blood pressure support scoring system" was used, as described earlier by our group (12): Score 1, volume expansion and/or dopamine $\leq$5 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Score 2, dopamine $>5 \leq 10$ $\mu g \cdot kg^{-1} \cdot min^{-1}$; Score 3, dopamine >10 $mg \cdot kg^{-1} \cdot min^{-1}$ or dopamine and dobutamine ≤ 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Score 4, dopamine and dobutamine >10 $\mu g \cdot kg^{-1} \cdot min^{-1}$; and Score 5, additional epinephrine and/or corticosteroids.

Diagnosis of RDS was made using clinical symptoms and radiographic signs as defined by Giedion et al. (3) and the need for artificial surfactant therapy. Decisions on surfactant replacement therapy were made by the attending neonatologist, who was unaware of the study results, on the basis of a defined protocol used in our unit. Diagnosis of PDA was made according to standard echocardiographic indexes.

Diagnosis of peri/intraventricular hemorrhage was performed using an ATL UM-4 mechanical sector scanner with a multifrequency transducer (5–7.5–10 MHz crystals).

Statistical analysis. Data are summarized as means (SD) or median and ranges where appropriate. Differences in clinical and laboratory data at the various time points between infants without RDS (no-RDS) or with RDS were assessed with Student's *t*-test, Mann-Whitney *U*-test, and χ^2 test, as appropriate. Differences in clinical and laboratory data to assess changes within groups over time were evaluated by ANOVA for repeated measurements, followed by Scheffé's procedure, if a significant difference was found.

A multiple linear regression model with dummy variables was used to investigate the respective contributions of plasma cGMP concentration and the existence of PDA to MABP changes using the following equation:

$$MABP = a_o + a_{cGMP}cGMP + a_{PDA}PDA + a_{cGMP \cdot PDA}cGMP \cdot PDA + a_{GA} \cdot GA + \sum a_{L,i} \cdot L_i \quad (1)$$

where MABP is the dependent variable, and a_o is its overall mean value. cGMP is the first independent variable and its coefficient a_{cGMP} defines the slope of the cGMP-MABP relationship. The second independent variable, PDA, is a dummy representing a closed (value -1) or persistent duct (value $+1$), and its coefficient a_{PDA} defines the slope of the PDA-MABP relationship. The third independent variable is an interaction variable, cGMP·PDA, representing the interactive effect of cGMP and a PDA on the MABP. The coefficient $a_{cGMP \cdot PDA}$ indicates, therefore, the effect of a PDA on the slope of the cGMP-MABP relationship. To investigate if gestational age (GA) has an independent effect on MABP in this cohort of preterm babies, GA was introduced as the fourth independent factor. Its coefficient a_{GA} defines the slope of the GA-MABP relationship. Finally, to correct for interpatient variability, 51 dummy variables (L_1-L_{51}) were introduced for the 52 patients included in this analysis. A detailed explanation of this statistical technique has been given elsewhere (4).

In a similar way, to investigate whether changes in CO and/or plasma NOx affected plasma cGMP concentration, a multiple linear regression model with dummy variables was used with the following equation:

$$cGMP = a_o + a_{COHb}COHb + a_{NOx}NOx + a_{COHb \cdot NOx}COHb \cdot NOx + a_{GA} \cdot GA + \sum a_{L,i} \cdot L_i \quad (2)$$

where cGMP is the dependent variable and a_o is its overall mean value. COHb and plasma NOx concentrations are the first and second independent variables, and their coefficients a_{COHb} and a_{NOx} define the slope of the COHb-cGMP and NOx-cGMP relationship, respectively. The third independent variable is an interaction variable, COHb·plasma NOx concentrations, representing the interactive effect of COHb and NOx on plasma cGMP concentration. The coefficient $a_{COHb \cdot NOx}$ indicates, therefore, the effect of NOx on the slope of the cGMP-COHb relationship. To investigate if GA has an independent effect on cGMP production, GA was introduced as the fourth independent factor. Its coefficient a_{GA} defines the slope of the GA-cGMP relationship. Finally, to correct for interpatient variability, 51 dummy variables (L_1-L_{51}) were introduced for the 52 patients included in this analysis.

Interpatient variability was quantified as the standard deviation of the set of patient coefficients.

To determine the statistical significance of any variable, an *F*-test was performed by dividing the mean square of that variable by the mean square residual.

To illustrate the individual relationships between blood COHb concentration and NOx on the one hand and plasma cGMP concentrations on the other, simple linear regressions were performed. The correlation coefficients are shown for descriptive purposes only. This was also done for the relationship between MABP and cGMP concentration. For statistical analysis, Statview II was used (Abacus Concepts, Berkeley, CA). Statistical significance was assumed for $P < 0.05$.

RESULTS

Fifty-two neonates were included in the study. Clinical characteristics are shown in Table 1. RDS was diagnosed in 31 infants. RDS infants had a significantly lower GA, but birth

Table 1. *Important clinical characteristics*

Clinical Data	No RDS (n = 21)	Yes RDS (n = 31)	P
Gestational age, wk (means ± SD)	30.2±1.5	29.1±1.7	<0.001
Birth wt, g (means ± SD)	1,235±310	1,238±423	
Apgar score (5 min) (median, range)	8 (6–10)	8 (2–10)	
Antepartum glucocorticoids, *n* (%)	18 (86)	19 (61)	<0.01
Assisted ventilation, *n* (%)	4 (19)	27 (87)	<0.001
CPAP, *n* (%)	6 (29)	4 (13)	
PDA, *n* (%)	2 (10)	14 (45)	<0.001
Time of first diagnosis, days (means ± SD)	2.4±2.1	2.1±1.4	
Peri/intraventricular hemorrhage, *n* (%)	3 (14)	9 (29)	<0.05
Plasma bilirubin, μmol/l (means ± SD)			
0–12 h	84±27	73±31	
48–72 h	111±28	116±47	
168 h	137±58	130±52	

See text for tests used for statistical analysis. Criteria for hemodynamically significant persistent ductus arteriosus (PDA) diagnosis given in text. RDS, respiratory distress syndrome; CPAP, continuous positive airway pressure;

weight or Apgar score did not differ between groups. Significantly more infants in the no-RDS group received glucocorticoids antenatally compared with infants with RDS. Most infants with RDS needed mechanical ventilation and were ventilated with higher mean airway pressures and for a longer period compared with infants without RDS (results not shown). PDA occurred more frequently in infants with RDS compared with those with no RDS. The average time of diagnosis of PDA was 2 days of age. Total plasma bilirubin values [mean (SD)] were not different between groups at any point in time (Table 1). Two infants with RDS eventually died.

Figure 1 shows plasma cGMP, COHb, and NOx values [mean (SD)] of the two groups of infants. Plasma cGMP levels were significantly higher in the RDS infants; this difference was significant at 48–72 h postnatally. COHb values were also higher in RDS infants during the study period, but significance was only reached at 48–72 and at 168 h postnatally. No differences were found between groups for plasma NOx values at any point in time. Within groups, COHb and cGMP were significantly higher at 48–72 h postnatally compared with 0–12 h or 168 h postnatally in the RDS group. This was not the case in the no-RDS infants, where only cGMP was significantly higher at 48–72 h compared with 168 h of age. To exclude a direct effect of antenatal glucocorticoids on COHb or cGMP, we compared COHb and cGMP concentrations between infants who received antenatal glucocorticoids and those who did not receive glucocorticoids within the respective groups (no-RDS and RDS). No differences were detected in any group (results not shown).

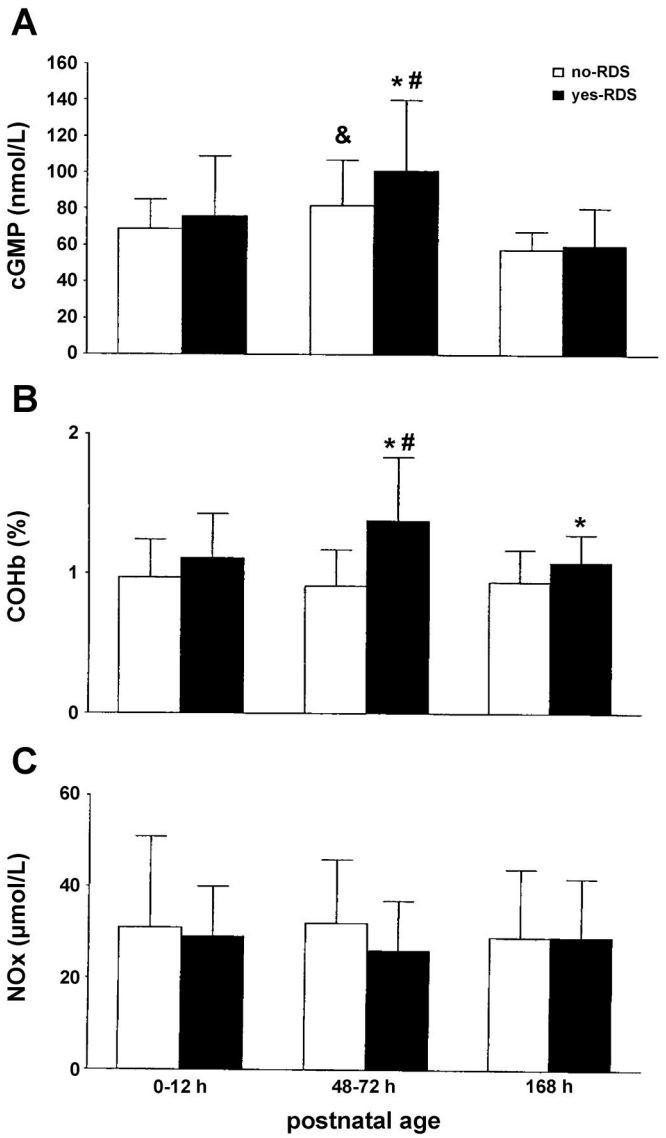

Fig. 1. cGMP (nmol/l) (*A*); carboxyhemoglobin (COHb; %) (*B*); and plasma levels of nitric oxide (NO) production (NOx; μmol/l) in plasma (*C*) [means (SD)] of infants without respiratory distress syndrome (no-RDS, $n = 21$) or with RDS (yes-RDS, $n = 31$), respectively, as a function of postnatal age. $^{*}P < 0.05$ vs no-RDS; $^{\#}P < 0.05$ vs. 0–12 h and 168 h yes-RDS; $^{\&}P < 0.05$ vs. 168 h no-RDS.

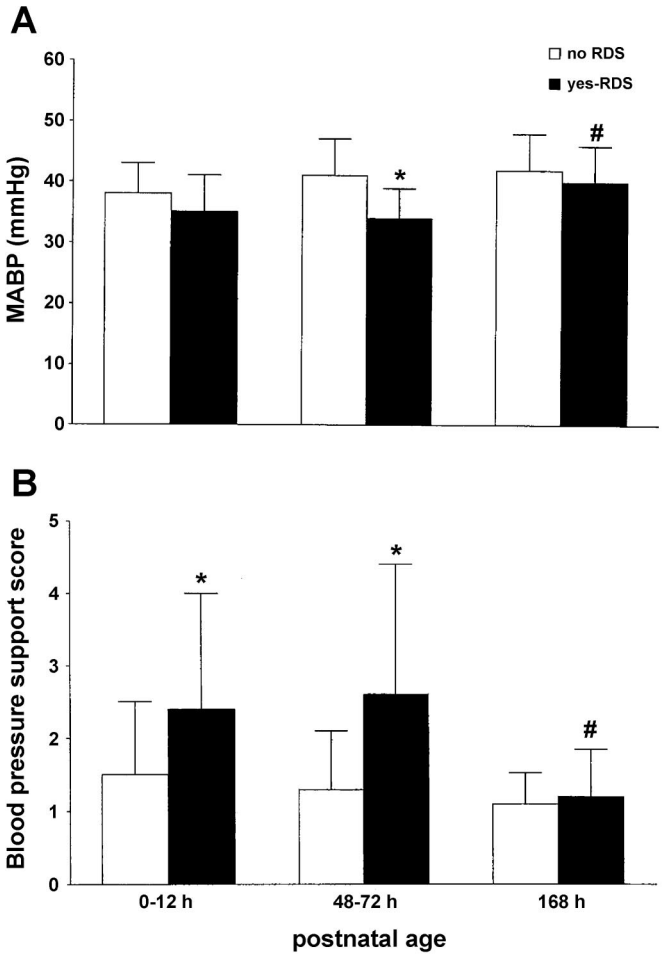

Fig. 2. Mean arterial blood pressure values (MABP; mmHg) (*A*) and blood pressure support score (*B*) [means (SD)] of no-RDS infants ($n = 21$) or yes-RDS infants ($n = 31$), respectively, as a function of postnatal age. $^{*}P < 0.05$ vs. no-RDS; $^{\#}P < 0.05$ vs 0–12 h (only *B*) and 48–72 h (*A* and *B*) yes-RDS.

MABP values and blood pressure support scores [mean (SD)] are shown in Fig. 2, *A* and *B*, as a function of postnatal age. MABP appeared to be significantly lower in RDS infants at 48–72 h of life, whereas the blood pressure support score was significantly higher at 48–72 h and at 168 h, respectively, in RDS infants compared with no-RDS infants. Only in the RDS infants was MABP significantly higher and blood pres-

Table 2. *Results of multiple linear regression analysis*

Independent Variables	Coefficients	*F* Values	F_{set}	*P*
	MABP			
cGMP	−0.08	5.59		0.02
PDA	0.94	0.05		0.83
cGMP*PDA	−0.01	0.21		0.66
GA	−0.96	0.07		0.80
Interpatient variability			0.72	0.38
	cGMP			
COHb	50.25	10.97		0.001
NOx	0.03	0.002		0.96
COHb*NOx	−0.32	0.58		0.45
GA	−0.088	0.27		0.61
Interpatient variability			1.54	

Results of multiple linear regression analyses of mean arterial blood pressure (MABP; dependent variable) on the independent variables: cGMP, PDA, cGMP*PDA (interaction variable), gestational age (GA); and on interpatient variability (*top*) and cGMP (dependent variable) on the independent variables: hemoglobin-bound CO (COHb), plasma nitric oxide (NOx), COHb*NOx (interaction variable), GA, and on interpatient variability *(bottom)*. Both regression equations were statistically significant ($P < 0.002$ and $P < 0.04$, respectively). F_{set} is the *F* value for the set of interpatient coefficients.

sure support score lower at 168 h compared with 0–12 h (score only) and 48–72 h postnatally.

The regression *equation 1* was statistically significant ($r = 0.70$, $P < 0.04$), showed a normal distribution, and revealed an inverse relationship between cGMP and MABP with an overall decrease of MABP of 0.08 mmHg per 1 nmol/l increase of plasma cGMP. Neither PDA nor GA had a significant independent effect on MABP. Interaction between cGMP and PDA also had no additional affect on MABP. Finally, no substantial interpatient variability could be found. The results are summarized in Table 2, *top*. For illustrative purposes only, the relationship between individual MABP values on the one hand and individual plasma cGMP concentrations on the other hand are depicted in Fig. 3 (MABP/cGMP values of infants, with PDA excluded: $r = -0.34$, $P < 0.001$; if all MABP/cGMP values were included: $r = -0.31$, $P < 0.001$, not shown).

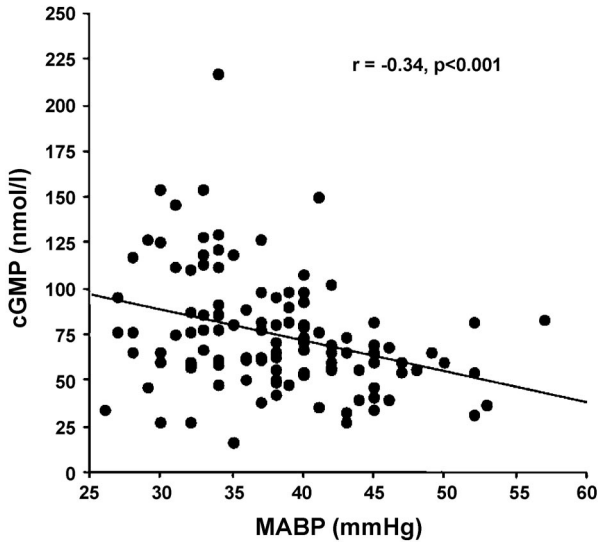

Fig. 3. Individual regression plots between MABP and cGMP values [values during patent ductus arteriosus (PDA) excluded].

The regression *equation 2* was also statistically significant ($r = 0.77$, $P < 0.002$), had a rather normal distribution, and revealed a positive relationship between cGMP and COHb with an overall increase of 50 nmol/l of plasma cGMP per 1% increase of COHb. No relationship was detected with NOx. Also, no interaction existed between plasma COHb and NOx in relation to cGMP production. GA did not play an independent role in cGMP production. Interpatient variability appeared to be minimal. The results are summarized in Table 2, *bottom*. For illustration purposes, only the individual relationship between plasma cGMP values on the one hand and COHb and NOx values on the other hand are depicted in Fig. 4, which shows the simple regression plots of cGMP as a function of COHb (*A*: $r = 0.35$, $P < 0.001$) and of cGMP as a function of NOx (*B*: $r = 0.04$, not significant).

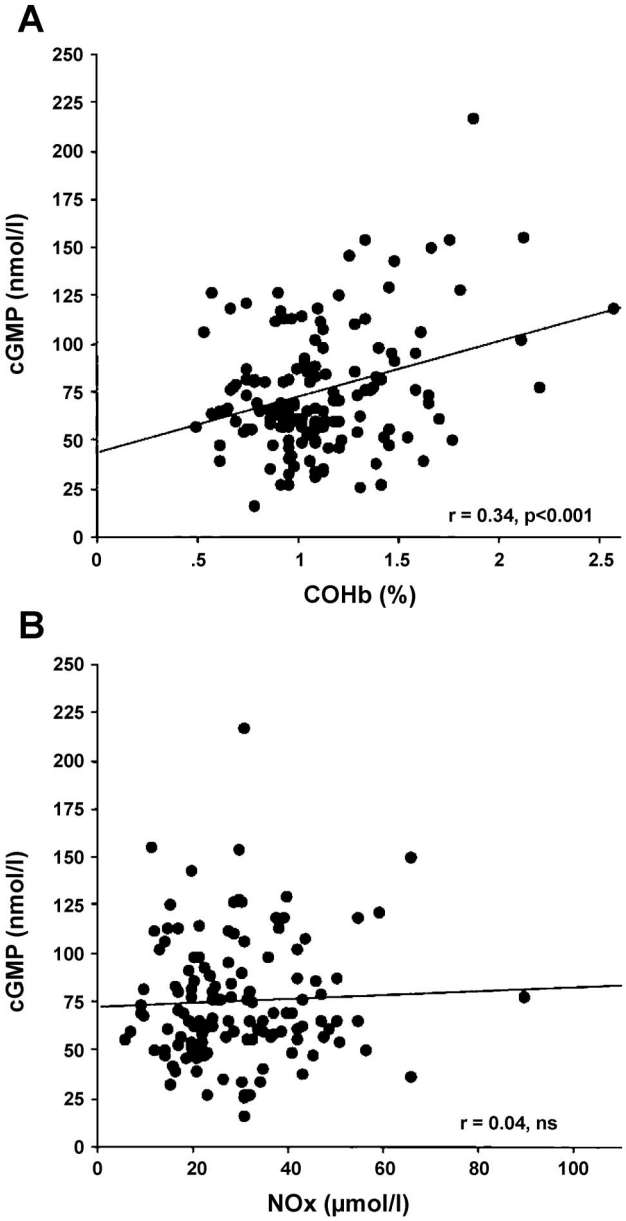

Fig. 4. Individual regression plots between plasma cGMP values and plasma COHb (*A*) or plasma NOx (*B*) values.

DISCUSSION

Consistently higher plasma cGMP levels were detected in infants with RDS, with the highest values being observed in infants with RDS at 48–72 h. Inasmuch as we found a similar pattern of COHb concentrations in plasma over postnatal time in this group and multiple linear regression revealed a significant relationship between COHb and cGMP only, we postulate a causal relationship between endogenous CO production and upregulation of cGMP. We further suggest that RDS, which involves inflammatory processes, significantly adds to total endogenous CO production, possibly due to an increased expression of inducible heme oxygenase. However, because no differences in plasma bilirubin concentrations could be found at any point of time between infants with or without RDS, other CO-producing processes, such as pulmonary or systemic lipid peroxidation, should not be ruled out as contributors to the body's CO pool.

Alternative pathways for activation of cGMP production should also be considered as an explanation for the observed results. These pathways include increased endogenous NOx production, a hypoxia-related increased production of the atrial natriuretic peptide, which has been shown to be increased significantly during the acute phase of RDS (7, 13), and, finally, a less efficient inactivation of cGMP by specific cyclic nucleotide phosphodiesterases (15). With respect to NOx production, this seems less probable as indicated by the results of an earlier study of our group (12, 18), together with the results of the present study that show no changes in NOx at any point in time in both groups and no relationship between NOx in plasma and cGMP plasma levels. Whether an atrial natriuretic peptide-related increase of cGMP or a less efficient inactivation of cGMP by specific cyclic nucleotide phosphodiesterases could have played a role here has not been investigated in the present study. However, with respect to a possible role of atrial natriuretic peptide, we can only point out that no important periods of hypoxia existed in any infant in our RDS group. We even considered the possibility that treatment of low blood pressures with dopamine and/or dobutamine directly affects plasma cGMP formation. However, we did not find any evidence in the literature for this mechanism. Finally, given the results, it seems unlikely that antenatal glucocorticoids had a direct effect on COHb and/or cGMP concentrations.

We therefore suggest that a CO-mediated upregulation of plasma cGMP levels in infants with RDS creates a state of increased vasodilation of the systemic arterial vascular bed. It was indeed shown that RDS infants tended to have lower MABPs during the first hours of life and significantly lower blood pressures at 48–72 h of life, despite rather intensive blood pressure support at that point of time (see also Fig. 2*B*). Moreover, the multiple linear regression analysis showed a clear inverse correlation between MABP and plasma cGMP concentrations, strongly suggesting a causal relationship between high cGMP production in the vascular bed on the one hand and low blood pressures and the need for antihypotensive therapy on the other. The above-suggested mediating effect of CO in the upregulation of plasma cGMP levels with a consequent negative effect on the blood pressure also suggests a relation between COHb levels and MABP, which was not the case. Probably, however, we are dealing here with an indirect effect of COHb on blood pressure in which cGMP acts as a mediator that transmits a significant portion of the functional effect of CO to blood pressure. Such indirect effects are also known as mediator effects in statistical models (10).

Although an increased cGMP level is surely not the only factor leading to lower blood pressure in the sick and unstable preterm infant suffering severe RDS, there is increasing evidence that it may add substantially to disturbances in blood pressure. Moreover, cGMP production-induced changes in arterial blood pressure and cerebral autoregulation seem to be associated with the development of moderate and severe periventricular/intraventricular hemorrhages that negatively influence later neuromotor development, as shown in an earlier study by our group (18). Future research on the prevention or reduction of oxidative stress and inflammation induced by RDS, for instance by antioxidative treatment (14, 16) and/or directly by reduction of cGMP levels in plasma in preterm infants with (severe) RDS (8), may be worthy of consideration.

In summary, we suggest that increased CO production in preterm infants with RDS, which is probably of pulmonary origin, contributed to increased cGMP plasma concentrations. This increase in cGMP may be an important contributor to the vasodilation-related low blood pressure seen during the acute phase of RDS in these preterm infants. Given the relatively small sample size of the present study and the importance of this common hemodynamic problem in preterm neonates, confirmation of our results may be important.

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