Available online at www.sciencedirect.com
ScienceDirect
Nitric Oxide 16 (2007) 448–456
NITRIC OXIDE Biology and Chemistry
www.elsevier.com/locate/yniox

Nitrite infusion increases cerebral blood flow and decreases mean arterial blood pressure in rats: A role for red cell NO

Joseph M. Rifkind [a,*], Enika Nagababu [a], Efrat Barbiro-Michaely [c], Somasundaram Ramasamy [a], Ryszard M. Pluta [b], Avraham Mayevsky [c]

[a] Molecular Dynamics Section, National Institute on Aging, 5600 Nathan Shock Drive, Baltimore, MD 21224, USA
[b] Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 10 Center Drive, Room 5D37, Bethesda, MD 20892-1662, USA
[c] The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University Ramat Gan, Israel

Received 27 December 2006; revised 14 March 2007
Available online 20 April 2007

Abstract

It has been proposed that the reduction of nitrite by red cells producing NO plays a role in the regulation of vascular tone. This hypothesis was investigated in rats by measuring the effect of nitrite infusion on mean arterial blood pressure (MAP), cerebral blood flow (CBF) and cerebrovascular resistance (CVR) in conjunction with the accumulation of red cell NO. The relative magnitude of the effects on MAP and CBF as well as the time dependent changes during nitrite infusion are used to distinguish between the effects on the peripheral circulation and the effects on the cerebral circulation undergoing cerebral autoregulation. The nitrite infusion was found to reverse the 96% increase in MAP and the 13% decrease in CBF produced by L-NAME inhibition of e-NOS. At the same time there was a 20-fold increase in oxygen stable red cell NO. Correlations of the red cell NO for individual rats support a role for red cell nitrite reduction in regulating vascular tone in both the peripheral and the cerebral circulation. Furthermore, data obtained prior to treatment is consistent with a contribution of red cell reduced nitrite in regulating vascular tone even under normal conditions.
© 2007 Published by Elsevier Inc.

Keywords: Cerebral circulation; Erythrocytes; Nitric oxide; Nitrite; Vasodilation

Nitric oxide (NO) synthesized from L-arginine by endothelial nitric oxide synthase plays an important role in regulating vascular tone and maintaining blood flow [1]. NO has a short half-life and needs to be produced/released in close proximity to the site were it reacts. Recent data reports that the reduction of nitrite to NO may play an important role in supplying NO for the regulation of blood flow [2,3].

Although mammals do not possess enzymes specifically designed for nitrite reduction, it has been shown that nitrite is reduced to NO in mammals. Nitrite can be reduced by mitochondria [4] as well as by xanthine oxidase [5,6]. However, most of the recent interest in nitrite reduction has emphasized the reduction by deoxygenated hemoglobin [2,3,7–12] that is present in red blood cells (RBCs) as oxygen is being delivered to the tissues. A physiological role for RBC nitrite reduction in regulating blood flow is supported by the observation that nitrite infusion results in an increase in RBC NO in conjunction with improved forearm blood flow, inhibition of hypoxic pulmonary vasoconstriction and in the prevention of delayed cerebral vasospasm [3,13–15].

Earlier studies on improved blood flow associated with nitrite infusion have been limited to the peripheral circulation. The cerebral blood flow (CBF) is, however, highly regulated under conditions of changing blood pressure, changes in carbon dioxide concentration and varying neuronal activity [16] to maintain an adequate supply of glucose and oxygen for brain function. NO has also been shown to regulate CBF [17,18]. To determine whether nitrite infusion and the resultant production of NO by

* Corresponding author. Fax: +1 410 558 8397.
E-mail address: rifkindj@mail.nih.gov (J.M. Rifkind).

1089-8603/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.niox.2007.04.002

nitrite reduction can also influence tightly regulated CBF, we used a rat model.

CBF, mean arterial blood pressure (MAP), hemoglobin oxygenation (HbO_2) and levels of mitochondrial NADH were measured in a group of rats prior to any treatment, after injection of L-NAME to inhibit nitric oxide synthase and during the subsequent infusion of nitrite. To elucidate a possible role for RBC-associated nitrite reduction, we have measured the nitrite-induced changes in the levels of RBC-NO and their correlation with the observed physiological changes.

Experimental procedures

Animal preparation and experimental design

All experiments were performed in accordance with the Animal Care Committee of Bar-Ilan University Guidelines. A total of 10 male Wistar rats of 250–300 g ($2\frac{1}{2}$–3 months old) were used. Two of these rats were controls, which were not injected with L-NAME or infused with nitrite. One of the other rats received the L-NAME injection, but was not infused with nitrite, and for an additional animal the blood sample taken for analysis of RBC-NO after nitrite infusion was lost. The rats were anesthetized with Equithesin (0.3 ml/100 g body weight, i.p.) (each ml contains: pentobarbital 9.72 mg, chloral hydrate 42.51 mg, magnesium sulfate 21.25 mg, propylene glycol 44.34% w/v, alcohol 11.5% and distilled water). During the duration of the experiment steady anesthesia was maintained by 0.1 ml Equithesin injection every 30 m. Additionally, the rats were placed on a warming tray and body temperature was maintained at 37 °C. Polyethylene catheters were introduced into the femoral vein for drug administration and into the femoral artery for systemic MAP monitoring. The jugular vein was also cannulated for obtaining blood samples.

During craniotomy, the rat was placed on an operation table with a special mouth holder. A 2 mm diameter hole was drilled in the parietal bone, and the bone was removed (the dura matter remained intact). Two screws were attached to the parietal bone, for better fixation of the TSFR (Time Sharing Fluorometer Reflectometer) to the brain surface. The monitoring probe was placed on the brain cortex using a special micromanipulator and fixated by dental acrylic cement [19,20].

After a period of stabilization (which included a short interval of anoxia induced by pure nitrogen inhalation, to evaluate reliable monitoring), a control sample of 0.6 ml blood was withdrawn and L-NAME (50 mg/kg body wt i.v.) was injected. Approximately 10 min after L-NAME administration (when MAP became stabilized at an elevated value) a second sample of blood was taken followed by a 10 min period of nitrite infusion. The rate of $NaNO_2$ infusion was 1 μmol/kg body wt/min. At the end of the infusion period a third sample of blood was taken. CBF, MAP, HbO_2 and NADH were continuously monitored through the entire experimental period. For the control animals the injections of saline and the withdrawal of blood had no effect on the CBF or MAP.

Time sharing fluorometer reflectometer (TSFR)

Simultaneously monitoring of mitochondrial NADH and HbO_2 was accomplished using the TSFR. This device contains appropriate filters located on a wheel that rotates at a speed of 3000 rpm, which is much faster then the kinetics of physiological changes, providing essentially simultaneous monitoring of NADH and HbO_2 [21] (Fig. 1).

Mitochondrial NADH redox state (NADH)

The redox state of mitochondrial NADH is determined by the fluorescence of NADH (the oxidized NAD^+ is not fluorescent). This fluorescence is monitored from the surface of the tissue by passing an excitation beam at 366 nm from the fluorometer to the tissue via a bundle of quartz optical fibers. This light is absorbed by the NADH and re-emitted at 450 nm. The emitted fluorescent light at 450 nm, together with the reflected light at the excitation wavelength (366 nm), is transferred to the fluorometer via another bundle of fibers. The changes in the reflected light are correlated to changes in tissue blood volume and are used to correct the fluorescent signal for hemodynamic artifacts, which also affect the fluorescent signal at 450 nm. The changes in corrected fluorescence are normalized relative to calibrated signals for non-treated animals under normoxic conditions [19,20,22,23].

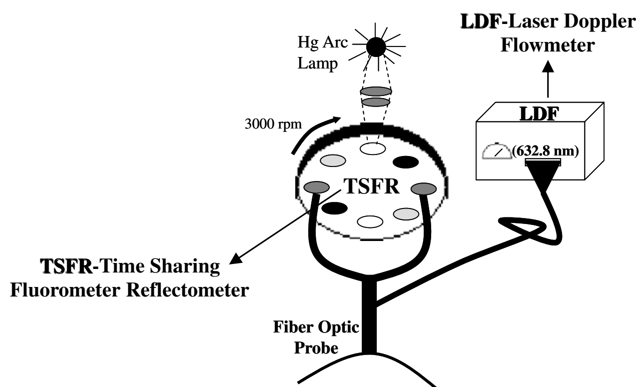

Fig. 1. A schematic presentation of the combination between the time sharing absorption and fluorescence probe (TSFR) and the fibers for blood flow monitoring (LDF) and its location on the brain cortex.

Tissue blood oxygenation

Tissue blood oxygenation was monitored based on the differences in the absorption properties of HbO_2 and deoxyhemoglobin. The tissue is illuminated with light at two wavelengths (585 and 577 nm). Five hundred eighty-five nanometer is an isosbestic point with the same extinction coefficient for HbO_2 and deoxyhemoglobin. The absorption at this wavelength reflects changes in tissue blood volume. At 577 nm, HbO_2 has a higher extinction coefficient than deoxyhemoglobin and by subtracting the 585 nm reflectance from the 577 nm reflectance a parameter correlated to blood oxygenation is obtained [21,24].

Cerebral blood flow

CBF was monitored by laser Doppler flowmetry (LDF), which measures relative changes in microcirculatory blood flow (0–100% range) [25]. This method was found to be highly correlated to the relative changes in tissue blood flow measured by the microshere method and H_2 clearance, which provide direct measures of blood flow [26–29]. The Doppler probe, which uses light of 632.8 nm, was included in the bundle of optical fibers used for the TSFR. The relative changes in cerebrovascular resistance (CVR) during nitrite infusion was determined from MAP/CBF [30].

Analysis of red cell NO

Blood samples were immediately centrifuged at 3000 rpm for 10 min and the plasma removed. The RBC pellet was stored in liquid nitrogen without further washing. NO was determined by a Nitric Oxide Analyzer (Sievers model 280) on thawed hemolysate as described earlier [2]. Total red cell NO was determined by lysing the cells in 4 volumes of deoxygenated distilled water in a septum sealed cuvette. Oxygen stable NO was determined by lysing the cells in 4 volumes of distilled water. About 100 μl of the sample was injected into the purge vessel containing 5.5 ml glacial acetic acid 20 mM sulfanilamide (to react with any free nitrite present) and 100 mM potassium ferricyanide (to release NO tightly bound to Fe(II) hemoglobin). While sulfanilamide reacts with free nitrite, it may not

react with nitrite bound to deoxyhemoglobin or methemoglobin. The released NO was flushed through the NO chemiluminescence analyzer and quantitated.

Statistical analysis

Origin 6.1 (Microcal Software, Northhampton MA) was used for analysis of the data. The paired Student's t-test was used for comparing samples before treatment with samples after L-NAME injection and after nitrite infusion. Linear regression analysis was used to compare parameters for the different rats studied. Two tailed values of $p < 0.05$ were considered statistically significant.

Results

The effect of nitrite infusion on vasoactivity in rats

There is a biphasic response of MAP to nitrite infusion. Within 1 min the MAP decreases by ~33% followed by a plateau till 4 min and a second drop, which levels off at 8 min (Fig. 2a). This biphasic response is not resolved for CBF (Fig. 2a) with no significant changes detected for the first 6 min. After 6 min the CBF begins to increase with a significant increase relative to the starting value observed as the 10 min infusion time approaches ($p < 0.02$). Comparing Fig. 2a and b it is noted that nitrite affects MAP more rapidly than CBF with the decrease in MAP leveled off when the most significant increase in CBF takes place.

Using the relative values of CBF obtained by LDF, it was also possible to calculate from MAP/CBF a relative value of CVR during nitrite perfusion (Fig. 2c). The CVR parallels for the most part the changes in MAP. The only difference is found for the last few minutes of the infusion where the increasing CBF, further lowers the CVR.

In order to evaluate the effect of nitrite infusion on the CBF it was necessary to use the CBF value obtained at the end of the nitrite infusion (the last point in Fig. 2a). For comparison, we therefore used the values obtained just before the blood-draw for both CBF and MAP.

L-NAME injection did not produce a significant change in CBF, although the infusion of nitrite resulted in a significant ($p < 0.05$) 28% increase in CBF (Fig. 3a). The changes in MAP were much larger (Fig. 3b) with the systemic MAP measured in the femoral artery almost doubled 10 min after NO synthesis inhibition by L-NAME ($p < 0.001$) and nitrite infusion for 10 min resulting in the complete reversal of the blood pressure increase ($p < 0.001$).

As a measure of tissue oxygenation, the multiparametric monitoring system was used to measure HbO_2 and mitochondrial NADH. Relatively minor changes in NADH and HbO_2 were detected (Fig. 4a and b). L-NAME caused a 2.9% increase in NADH (Fig. 4a), which was not statistically significant. After nitrite infusion a significant 5.6% increase in NADH was observed relative to the value obtained after NO synthase inhibition ($p < 0.05$). No significant changes were observed in HbO_2 either after L-NAME or nitrite (Fig. 4b). Both of these results rule out any major changes in tissue oxygenation.

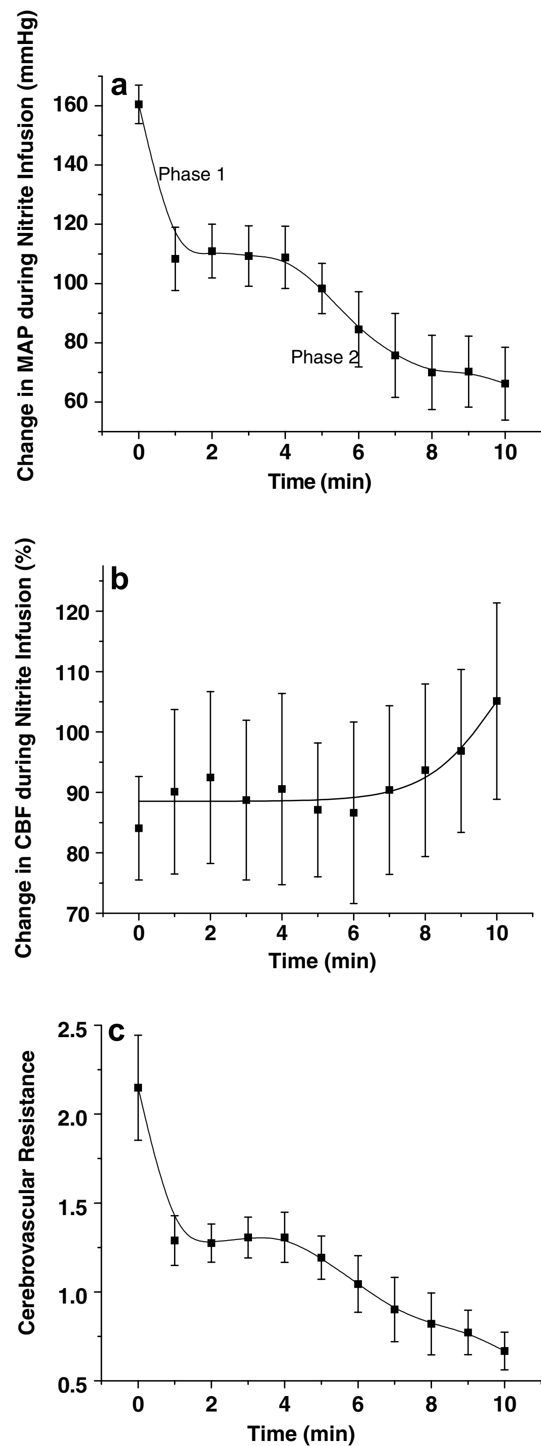

Fig. 2. Changes in the various parameters during the 10 min nitrite infusion. The error bars indicate the standard error. This infusion was started 10 min after the injection of L-NAME. (a), the mean arterial blood pressure (MAP); (b), cerebral blood flow (CBF); (c), the relative cerebrovascular resistance (CVR) calculated as MAP/CBF.

The effect of nitrite infusion on red cell nitric oxide

Table 1 summarizes the effect of L-NAME and nitrite on the total chemiluminescence signal when the sample was lysed in deoxygenated buffer, and for the oxygen stable

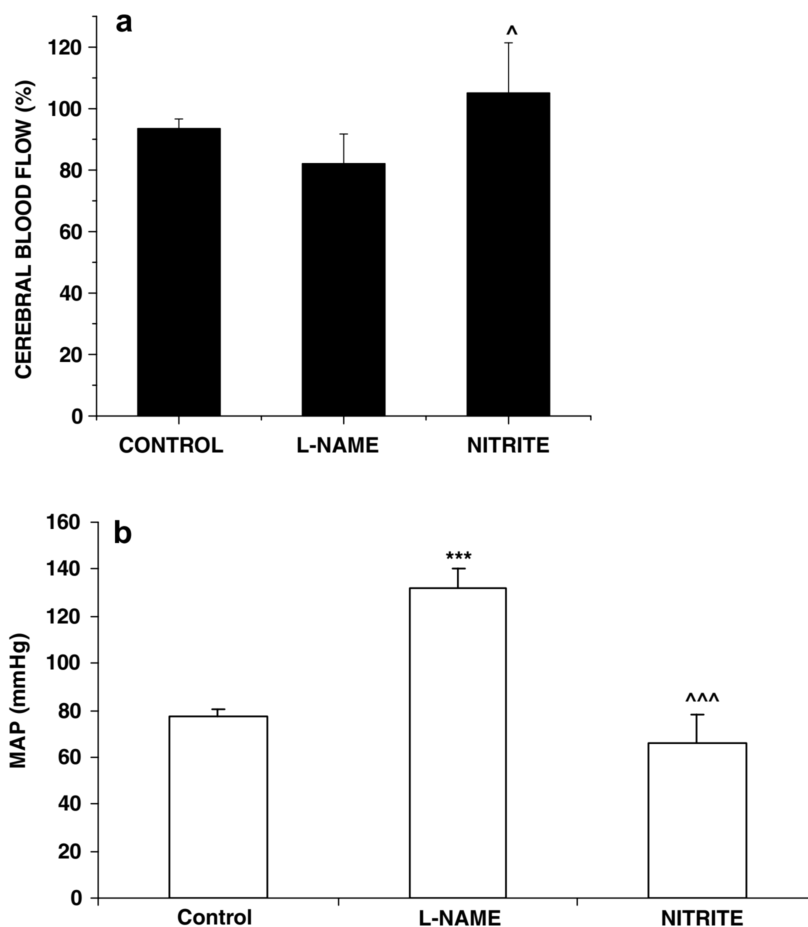

Fig. 3. Values for CBF and MAP. The error bars indicate the standard error. (a) Average values of cerebral blood flow (CBF) prior to treatment, after L-NAME injection and after nitrite infusion. Values for each rate is given relative to an initial value of each rat set at 100.^ indicates that the value is significantly different from the L-NAME results ($p < 0.05$). (b) Average values of mean arterial blood pressure (MAP) prior to treatment, after L-NAME injection and after nitrite infusion. *** indicates that the value is significantly different from the control results ($p < 0.001$); ^^^ indicates that the value is significantly different from the L-NAME results ($p < 0.001$).

NO chemiluminescence signal (the signal observed with the sample lysed in oxygenated buffer). While L-NAME did not produce any significant changes in RBC NO, the 10 min infusion with nitrite produced a dramatic 20-fold increase in the oxygen stable RBC NO ($p < 0.0001$) and a 40-fold increase in the total RBC NO ($p < 0.0001$). Under all conditions the total RBC NO was significantly greater than the oxygen stable RBC NO. For the control only 41% of the NO was stable in oxygen ($p < 0.001$), after L-NAME injection 48% of the NO was stable in oxygen ($p < 0.001$) and after nitrite infusion 52% was stable in oxygen ($p < 0.05$). This trend is similar to that found for human blood [2] and is consistent with most of the red cell NO originating from nitrite reduction even prior to nitrite infusion.

Relationship between red cell NO and vasoactivity

To investigate a possible relationship between RBC NO and vasoactivity it was possible to utilize the variability in the level of RBC NO among the rats studied. When the total RBC NO was used for this purpose (data not shown), there was no significance, which can be attributed to the instability of the oxygen labile RBC NO resulting in a partial variable loss of this NO during sample preparation.

It was, however, possible to delineate a potential contribution of RBC NO to nitrite-induced cerebral and peripheral vasoactivity (see below), by using the oxygen stable RBC NO of each rat studied. Significant correlation were obtained with oxygen stable RBC NO on the nitrite induced change in relative CVR (Fig. 5a; $r = -0.843$; $p < 0.05$), which is a measure of cerebral vasoactivity and the nitrite induced change in MAP (Fig. 5b; $r = -0.83$; $p < 0.05$), which is a measure of peripheral vasoactivity.

To investigate a possible relationship between basal RBC NO and vasoactivity, CBF, and therefore CVR, could not be used to compare results on different rats, because LDF only provides a relative value of blood flow. A role for RBC NO in the absence of added nitrite is, however, suggested by the significant positive correlation between basal oxygen stable RBC NO and MAP ($r = 0.853$; $p < 0.05$) (Fig. 6).

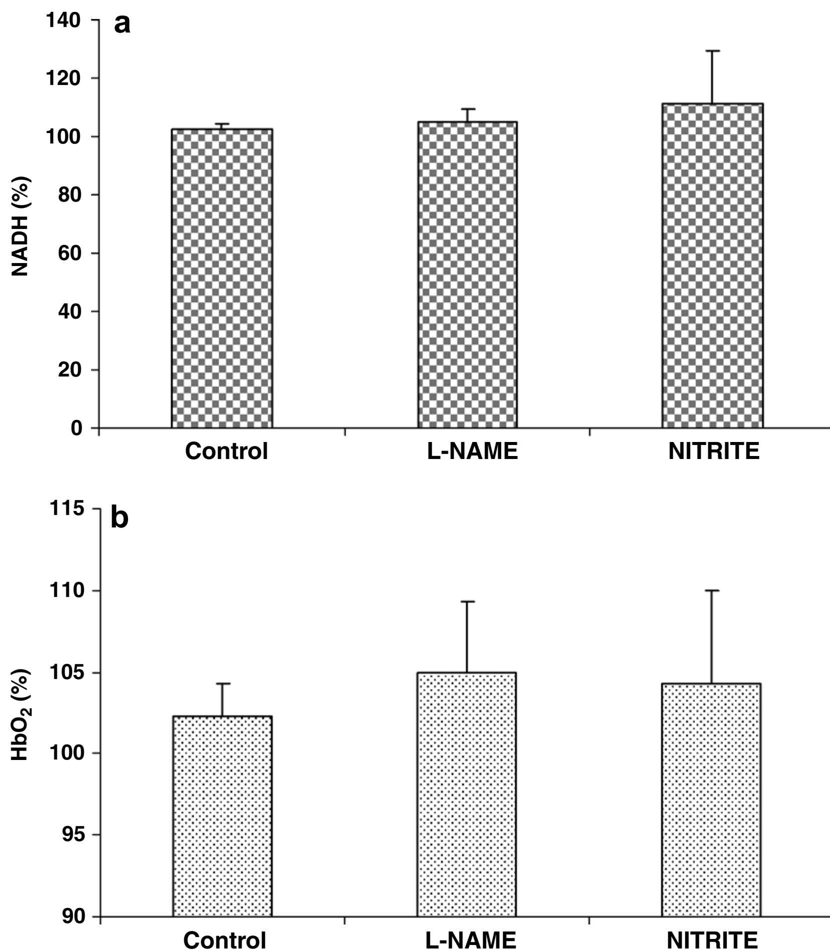

Fig. 4. Relative values for NADH and hemoglobin oxygenation (HbO_2). The error bars indicate the standard error (a) Average values of NADH prior to treatment, after L-NAME injection and after nitrite infusion. Values for each rat is given relative to an initial value of each rat set at 100 (b). Average values of HbO_2 prior to treatment, after L-NAME injection and after nitrite infusion. Values for each rate is given relative to the initial value of each rat set at 100.

Table 1
Red cell Nitric Oxide

Sample	Total RBC NO (μM)	Oxygen stable RBC NO (μM)
Control	0.83 ± 0.09[a]	0.40 ± 0.09
L-NAME	1.30 ± 0.23	0.53 ± 0.13
Nitrite	40.33 ± 19.64	21.17 ± 6.49

[a] Standard error of the means.

Discussion

The red cell nitric oxide

It has been previously demonstrated that, during the reaction of nitrite with deoxyhemoglobin, oxygen labile and oxygen stabile chemiluminescent intermediate species are observed in addition to the Fe(II) heme complex detected by electron paramagnetic resonance [10]. These intermediates have not been fully characterized and efforts to characterize them are on-going. These intermediates should, however, include the delocalized species with an electron shared between the iron, the NO and perhaps the β-chain thiol [2,10,31] as well as any nitrite or nitrite metabolite associated with deoxyhemoglobin or methemoglobin, which can be converted to NO during the chemiluminescence assay. It is also possible that the oxygen labile pool can even include non-bound nitrite in the red cell [32] that reacts with hemoglobin in the deoxygenated buffer prior to being assayed.

Irrespective of the nature of these intermediates, the presence of an oxygen labile and oxygen stable chemiluminescent signal is indicative of the involvement of nitrite reduction. Thus, iron-nitrosyl-hemoglobin only gives an oxygen stable chemiluminescent signal that is also detected by electron paramagnetioc resonance and S-nitosylated hemoglobin is not detected at all unless the NO is first released from the thiol by copper [2,31].

The presence of an oxygen labile and an oxygen stable chemiluminescence signal in human blood samples with the labile pool corresponding to ~75% of the total RBC NO was, thus, previously attributed to an *in vivo* reaction

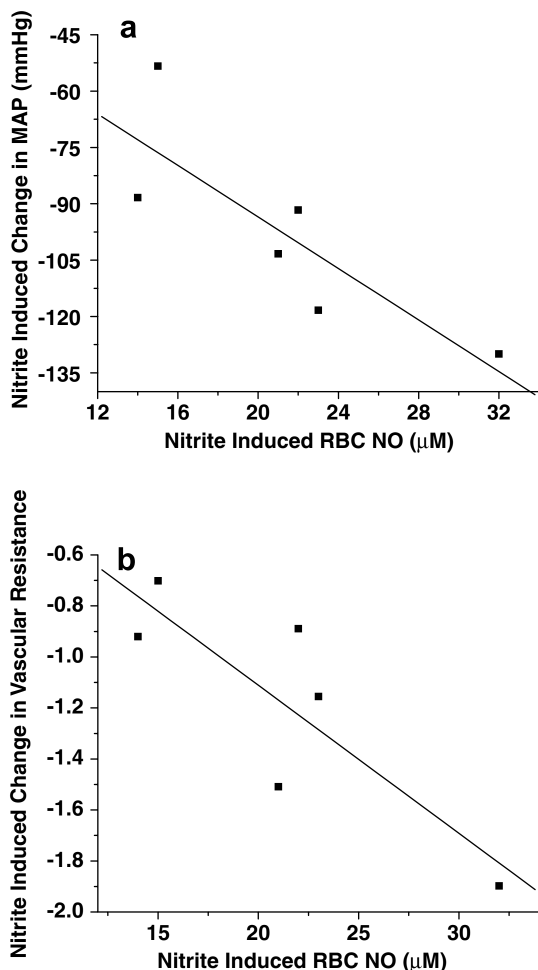

Fig. 5. Relationship with oxygen stable red cell nitric oxide after nitrite infusion. (a) Mean arterial blood pressure (MAP); (b) cerebrovascular resistance (CVR).

of nitrite with deoxygenated red cells [2]. In the current study we confirm the presence of these two pools of NO during nitrite infusion. We find (Table 1) that after nitrite infusion only ~52% of the RBC NO formed (~21 μM) is in the oxygen stable form with 19 μM NO lost when the sample is lysed in oxygenated buffer representing the oxygen labile pool of NO.

Prior to nitrite infusion (Table 1), both for the basal samples without any treatment and after L-NAME injection, more than 50% of the RBC NO is in the labile pool. This observation indicates that a major fraction of basal RBC NO originates from nitrite reduction.

The dramatic increase in RBC NO after only 10 min infusion (Table 1) is indicative of the efficiency of nitrite reduction by RBCs *in vivo*. The ~40 μM total RBC NO corresponds to ~10% of the 10 μmole/kg total nitrite infused into the rat[1]. This actually underestimates the

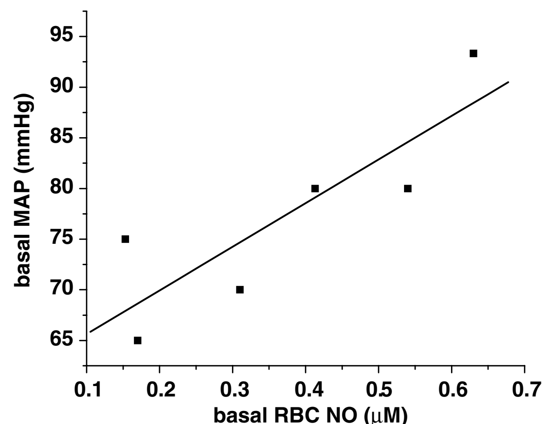

Fig. 6. Relationship between mean arterial blood pressure (MAP) and oxygen stable red cell nitric oxide prior to any treatment.

nitrite reduced to NO, both because of the utilization of some of this NO to modulate blood pressure and blood flow and the possible loss of labile NO during sample preparation. The efficiency of RBC nitrite reduction is further indicated by the fact that the infused nitrite needs to be transported into the RBC and react with the intracellular hemoglobin. At the same time the nitrite comes in contact with other cells, which can also interact with the nitrite.

Nitrite induced vasodilatation

In conjunction with the dramatic increase in RBC NO detected after nitrite infusion (Table 1), the L-NAME effects on CBF and MAP are reversed (Fig. 3a and b). These changes indicate an effect of nitrite on both the peripheral circulation and the cerebral circulation. While this vasodilation could be associated with the RBC NO, it is necessary to consider other ways that nitrite infusion can cause vasodilation and influence vascular tone.

An effect of nitrite on MAP, CBF and CVR can be directly mediated by hypoxia resulting from a reduction in oxygen carrying capacity of blood caused by nitrite induced hemoglobin oxidation [33,34]. The absence of an appreciable increase in NADH and decrease in HbO_2 (Fig. 4), rules out a dominant role for hypoxia.

Although mammals do not possess enzymes known to be specifically designed for nitrite reduction, multiple potential pathways for nitrite reduction in mammals have been discussed [12]. Xanthine oxidase can reduce nitrite to NO [5,6]. Nitrite can be reduced by cobalamin [35] or other heme proteins such as cytoglobin and myoglobin [7] and nitrite can even be reduced to NO without the involvement of a protein at reduced pHs [36]. A contribution from nitrite reduction by one of these processes cannot be ruled out. In fact the biphasic nitrite effect on MAP (Fig. 2a) implies that more than one process is involved in nitrite induced vasodilation. Nevertheless, the observation that a major fraction of the infused nitrite is found

[1] For a 250 g rat blood volume = 0.064 × 250 = 16 × 0.4 hct = 6.4 ml red blood cells = 0.0064 L × 40 μmole/l = 0.256 μmole which more than 10% of the 2.5 μmole/250 g rat infused.

in the RBC is consistent with a role for the RBC in reversing the L-NAME vasoconstriction.

By utilizing the level of RBC NO in each rat studied, a role for RBC NO in both the peripheral circulation and the cerebral circulation is implied. MAP measured in the peripheral circulation is determined by changes in the overall vasoconstriction and/or vasodilation, which directly affect the blood pressure. The correlation of MAP with oxygen stable RBC NO (Fig. 5a) is, thus, consistent with RBC NO regulation of peripheral vascular tone when the RBC reacts with nitrite after L-NAME inhibition of e-NOS. An analogous relationship between oxygen stable red cell NO metabolites and peripheral blood pressure and blood flow has previously been reported [3].

Vasoconstriction or vasodilation limited to the cerebral vasculature accounts for a relatively small fraction of the total blood volume [37], and will have only a minor affect on MAP. The MAP, which contributes to the cerebral perfusion pressure together with the intracranial pressure [38], however provides the force driving blood through the cerebral circulation [39]. A decrease in MAP is, thus, expected to decrease CBF [37,40,41] although autoregulation will at least partially compensate for any drop in CBF. Since the CBF includes factors, which reflect changes in the cerebral vasculature as well as changes in MAP, unrelated to any change in the vasculature, a more valuable measure of changes in the cerebral circulation is provided by CVR [18,40] obtained from the ratio MAP/CBF. Eventhough LDF only provides a relative value of CBF, a meaningful value of the change in CBF obtained after nitrite perfusion can be used to provide a relative value of CVR [30]. The significant negative correlation between this nitrite induced change in CVR and oxygen stable RBC NO (Fig. 5b) suggests a role for RBC NO in regulating the cerebral circulation.

Our results on rats before any treatment further implicate a role for red cell nitrite reduction under basal conditions when nitrite is not being infused. The finding that ~50% of the red cell NO is in the labile form even prior to nitrite infusion (Table 1) indicates that nitrite reduction accounts for a major fraction of the basal red cell NO. The significant correlation of the basal RBC NO with MAP (Fig. 6) furthermore implies that this RBC NO seems to play a role in the regulation of peripheral vascular tone. As indicated above for cerebral vascular tone, the involvement of RBC NO post nitrite infusion is demonstrated. However, the possible effect on basal cerebral vascular tone prior to nitrite infusion cannot be addressed, because the CBF obtained from LDF is only a relative measure of cerebral blood flow making it impossible to compare basal levels for individual rats.

It is necessary to explain the opposite relationship with MAP of oxygen stable RBC NO under basal conditions (Fig. 6) and after nitrite infusion (Fig. 5a). This apparent contradiction is explained by the difference between an acute infusion of nitrite and the basal pseudo-equilibrium conditions present prior to any treatment. Under basal conditions a lower level of oxygen stable RBC NO is obtained when a greater fraction of the red cell NO is utilized by the vasculature lowering the MAP. However, during nitrite infusion the level of oxygen stable RBC NO formed reflects the amount of nitrite being reduced and, therefore, the availability of RBC NO for the vasculature.

These results support the hypothesis that bioactive red cell NO produced either during nitrite infusion or by the reduction of nitrite already in the plasma plays a role in fine tuning blood flow in the peripheral circulation and at least after nitrite perfusion it also fine tunes the cerebral circulation.

Comparison between the response to nitrite of the peripheral circulation and the cerebral circulation

The finding that the cerebral circulation is affected by nitrite infusion is of particular interest, because the cerebral circulation is highly regulated by cerebral autoregulation [16,39,41], which is designed to maintain constant CBF to assure the regulated delivery of oxygen and nutrients to the brain.

A contribution of cerebral autoregulation, which minimizes the changes of CBF when MAP, the primary driving force for blood flow through the cerebral circulation, increases or decreases is evident by comparing the effect of L-NAME and nitrite on CBF and MAP (Fig. 3a and b). Thus, although L-NAME produces a 96% increase in MAP, e-NOS inhibition by L-NAME produces only an insignificant 13% decrease in CBF. The subsequent infusion with nitrite completely reverses the 96% increase in MAP, but only increases CBF by 28%.

The time course for nitrite induced changes in CBF and MAP after e-NOS is inhibited by L-NAME (Fig. 2) also demonstrates the contribution of cerebral autoregulation. The initial rapid drop in MAP decreases the force driving the blood through the capillary beds and should decrease the CBF. The constant CBF (Fig. 2a) during the first 7 min when the MAP is decreasing demonstrates cerebral autoregulation, including a contribution from NO induced vasodilatation of the cerebral blood vessels increasing blood flow. The drop in CVR, during this time frame (Fig. 2d), indicates changes to the cerebral vasculature, associated with cerebral autoregulation. These results can be compared with the previously reported [3] study of the effect of nitrite perfusion on the peripheral circulation. In the absence of cerebral autoregulation a 175% increase in forearm blood flow was observed, which was actually greater than the observed change in blood pressure.

In the cerebral circulation NO cannot produce an increase in the CBF until it can overcome the cerebral autoregulation that minimizes any change in CBF. The smaller percent change in CBF than MAP (Fig. 3) does, therefore, not indicate the relative utilization of NO by the peripheral and cerebral circulation. The time dependent changes for CBF and MAP actually support greater utilization of NO by the cerebral circulation than the peripheral circulation. Thus, the

CBF increases during the last 3 min of infusion, when the MAP is no longer responsive to the additional nitrite.

The regulation of cerebral blood flow is essential to maintain an adequate supply of oxygen for neuronal function. These results establish a contribution of nitrite, and possibly red cell reduction of nitrite to NO, to the highly regulated cerebral blood flow.

Acknowledgments

This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging and the National Institute of Neurological Disorders and Stroke. This research was also partially supported by The Israel Science Foundation Grant No. 358/04.

References

[1] L.J. Ignarro, G. Cirino, A. Casini, C. Napoli, Nitric oxide as a signaling molecule in the vascular system: an overview, J. Cardiovasc. Pharmacol. 34 (1999) 879–886.

[2] E. Nagababu, S. Ramasamy, D.R. Abernethy, J.M. Rifkind, Active nitric oxide produced in the red cell under hypoxic conditions by deoxyhemoglobin-mediated nitrite reduction, J. Biol. Chem. 278 (2003) 46349–46356.

[3] K. Cosby, K.S. Partovi, J.H. Crawford, R.P. Patel, C.D. Reiter, et al., Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation, Nat. Med. 9 (2003) 1498–1505.

[4] H. Nohl, K. Staniek, B. Sobhian, S. Bahrami, H. Redl, A.V. Kozlov, Mitochondria recycle nitrite back to the bioregulator nitric monoxide, Acta Biochim. Pol. 47 (2000) 913–921.

[5] T.M. Millar, C.R. Stevens, N. Benjamin, R. Eisenthal, R. Harrison, D.R. Blake, Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions, FEBS Lett. 427 (1998) 225–228.

[6] H. Li, A. Samouilov, X. Liu, J.L. Zweier, Characterization of the magnitude and kinetics of xanthine oxidase-catalyzed nitrite reduction. Evaluation of its role in nitric oxide generation in anoxic tissues, J. Biol. Chem. 276 (2001) 24482–24489.

[7] Z. Huang, S. Shiva, D.B. Kim-Shapiro, R.P. Patel, L.A. Ringwood, et al., Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control, J. Clin. Invest. 115 (2005) 2099–2107.

[8] K.T. Huang, A. Keszler, N. Patel, R.P. Patel, M.T. Gladwin, et al., The reaction between nitrite and deoxyhemoglobin. Reassessment of reaction kinetics and stoichiometry, J. Biol. Chem. 280 (2005) 31126–31131.

[9] C.J. Roche, D. Dantsker, U. Samuni, J.M. Friedman, Nitrite reductase activity of sol-gel-encapsulated deoxyhemoglobin. Influence of quaternary and tertiary structure, J. Biol. Chem. 281 (2006) 36874–36882.

[10] J.M. Rifkind, E. Nagababu, S. Ramasamy, Nitric oxide redox reactions and red cell biology, Antioxid. Redox Signal 8 (2006) 1193–1203.

[11] M.P. Doyle, R.A. Pickering, T.M. DeWeert, J.W. Hoekstra, D. Pater, Kinetics and mechanism of the oxidation of human deoxyhemoglobin by nitrites, J. Biol. Chem. 256 (1981) 12393–12398.

[12] V.P. Reutov, E.G. Sorokina, NO-synthase and nitrite-reductase components of nitric oxide cycle, Biochemistry (Mosc) 63 (1998) 874–884.

[13] S. Deem, J.H. Min, J.D. Moulding, R. Eveland, E.R. Swenson, Red blood cells prevent inhibition of hypoxic pulmonary vasoconstriction by nitrite in isolated, perfused rat lungs, Am. J. Physiol. Heart Circ. Physiol. (2006).

[14] J.H. Crawford, T.S. Isbell, Z. Huang, S. Shiva, B.K. Chacko, et al., Hypoxia, red blood cells, and nitrite regulate NO-dependent hypoxic vasodilation, Blood 107 (2006) 566–574.

[15] R.M. Pluta, A. Dejam, G. Grimes, M.T. Gladwin, E.H. Oldfield, Nitrite infusions to prevent delayed cerebral vasospasm in a primate model of subarachnoid hemorrhage, JAMA 293 (2005) 1477–1484.

[16] O.B. Paulson, S. Strandgaard, L. Edvinsson, Cerebral autoregulation, Cerebrovasc. Brain Metab. Rev. 2 (1990) 161–192.

[17] J.E. Buchanan, J.W. Phillis, The role of nitric oxide in the regulation of cerebral blood flow, Brain Res. 610 (1993) 248–255.

[18] B.G. Thompson, R.M. Pluta, M.E. Girton, E.H. Oldfield, Nitric oxide mediation of chemoregulation but not autoregulation of cerebral blood flow in primates, J. Neurosurg. 84 (1996) 71–78.

[19] A. Mayevsky, B. Chance, Intracellular oxidation-reduction state measured in situ by a multichannel fiber-optic surface fluorometer, Science 217 (1982) 537–540.

[20] A. Mayevsky, Brain NADH redox state monitored in vivo by fiber optic surface fluorometry, Brain Res. 319 (1984) 49–68.

[21] I.J. Rampil, L. Litt, A. Mayevsky, Correlated, simultaneous, multiple-wavelength optical monitoring in vivo of localized cerebrocortical NADH and brain microvessel hemoglobin oxygen saturation, J. Clin. Monit. 8 (1992) 216–225.

[22] A. Mayevsky, Brain energy metabolism of the conscious rat exposed to various physiological and pathological situations, Brain Res. 113 (1976) 327–338.

[23] A. Mayevsky, R.B. Duckrow, E. Yoles, N. Zarchin, D. Kaushausky, Brain mitochondrial redox state, tissue haemodynamic and extracellular ion responses to four-vessel occlusion and spreading depression in the rat, Neurol. Res. 12 (1990) 243–248.

[24] K.H. Frank, M. Kessler, K. Appelbaum, W. Dummler, The Erlangen micro-lightguide spectrophotometer EMPHO I, Phys. Med. Biol. 34 (1989) 1883–1900.

[25] K. Wadhwani, S.I. Rapoport, Blood flow in the central and peripheral nervous systems, in: A.P. Shephard, P.A. Oberg (Eds.), Lase Doppler Blood Flowmetry, Kluwer Academic Publishers, Boston, 1996, pp. 265–304.

[26] R. Hickey, M.S. Albin, L. Bunegin, J. Gelineau, Autoregulation of spinal cord blood flow: is the cord a microcosm of the brain? Stroke 17 (1986) 1183–1189.

[27] M.C. Wallace, C.H. Tator, Spinal cord blood flow measured with microspheres following spinal cord injury in the rat, Can. J. Neurol. Sci. 13 (1986) 91–96.

[28] C. Werner, W.E. Hoffman, E. Kochs, J. Schulte am Esch, R.F. Albrecht, The effects of propofol on cerebral and spinal cord blood flow in rats, Anesth. Analg. 76 (1993) 971–975.

[29] W. Reith, M. Forsting, J. Weber, R. Stingele, W. Hacke, K. Sartor, Laser Doppler flowmetry of focal ischaemia and reperfusion in deep brain structures in rats, Acta Neurochir. (Wien) 131 (1994) 151–156.

[30] K. Chida, M. Miyagawa, W. Usui, H. Kawamura, T. Takasu, K. Kanmatsuse, Effects of chemical stimulation of the rostral and caudal ventrolateral medulla on cerebral and renal microcirculation in rats, J. Auton. Nerv. Syst. 51 (1995) 77–84.

[31] E. Nagababu, S. Ramasamy, J.M. Rifkind, S-nitrosohemoglobin: A mechanism for its formation in conjuction with nitrite reduction by deoxyhemoglobin, Nitric Oxide 15 (2006) 20–29.

[32] A. Dejam, C.J. Hunter, M.M. Pelletier, L.L. Hsu, R.F. Machado, et al., Erythrocytes are the major intravascular storage sites of nitrite in human blood, Blood 106 (2005) 734–739.

[33] R. Klimmek, C. Roddewig, H. Fladerer, C. Krettek, N. Weger, Effects of 4-dimethylaminophenol, Co2EDTA, or NaNO2 on cerebral blood flow and sinus blood homeostasis of dogs in connection with acute cyanide poisoning, Toxicology 26 (1983) 143–154.

[34] R. Klimmek, C. Roddewig, N. Weger, Effects of 4-dimethylaminophenol on blood flow and blood gases in the brain, Res. Exp. Med. (Berl.) 179 (1981) 141–151.

[35] M. Wolak, A. Zahl, T. Schneppensieper, G. Stochel, R. van Eldik, Kinetics and mechanism of the reversible binding of nitric oxide to reduced cobalamin B(12r) (Cob(II)alamin), J. Am. Chem. Soc. 123 (2001) 9780–9791.
[36] J.L. Zweier, P. Wang, A. Samouilov, P. Kuppusamy, Enzyme-independent formation of nitric oxide in biological tissues, Nat. Med. 1 (1995) 804–809.
[37] P. Schumann, O. Touzani, A.R. Young, R. Morello, J.C. Baron, E.T. MacKenzie, Evaluation of the ratio of cerebral blood flow to cerebral blood volume as an index of local cerebral perfusion pressure, Brain 121 (Pt 7) (1998) 1369–1379.
[38] R. Hlatky, A.B. Valadka, C.S. Robertson, Intracranial pressure response to induced hypertension: role of dynamic pressure autoregulation, Neurosurgery 57 (2005) 917–923, discussion 917–923.
[39] J.M. Serrador, F.A. Sorond, M. Vyas, M. Gagnon, I.D. Iloputaife, L.A. Lipsitz, Cerebral pressure-flow relations in hypertensive elderly humans: transfer gain in different frequency domains, J. Appl. Physiol. 98 (2005) 151–159.
[40] I. Lartaud, L. Bray-des-Boscs, J.M. Chillon, J. Atkinson, C. Capdeville-Atkinson, In vivo cerebrovascular reactivity in Wistar and Fischer 344 rat strains during aging, Am. J. Physiol. 264 (1993) H851–H858.
[41] U. Dirnagl, W. Pulsinelli, Autoregulation of cerebral blood flow in experimental focal brain ischemia, J. Cereb. Blood Flow Metab. 10 (1990) 327–336.