Role of nitrite in neurovascular coupling: Nitric oxide-dependent and independent mechanisms

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INTRODUCTION:

In addition to its classic role and a vasodilator, nitric oxide (NO) acts as a neurotransmitter in neuronal tissue. Of particular interest is the coupling of these two functions in the brain response to various stimuli - the neurovascular coupling and the exact role of NO derived from neuronal nitric oxide synthase (nNOS) in it. [1]. It is known that inhibition of nNOS causes uncoupling between neuronal and hemodynamic responses to functional activation of the somatosensory pathway in α-chloralose anesthetized rats [2]. In the present study, we investigate whether systemic or topical administration of NO in form of sodium nitroprusside (SNP) restores neurovascular coupling. In addition, we also tested nitrite, an NO precursor. Nitrite is a therapeutically promising vasodilatory compound, as it is not only metabolized to NO, but also alters ATP release from erythrocytes [3] and can potentially increase H_2O_2 levels.

METHODS:

fMRI experiments: α-chloralose anesthetized Sprague-Dawley rats (n=7) were used. Experiments were carried out on a 7 T/30cm magnet (Bruker-Biospin, Billerica, MA). A dynamic arterial spin labeling (DASL) echo-planar imaging sequence (TR/TE 250/15 ms; 400x400x2000 μm3) was used to measure CBF and BOLD responses prior to and following an i.p. bolus (50 mg/kg) of 7-nitroindazole (7-NI, an *in vivo* inhibitor of nNOS), and after subsequent IV administration of SNP or nitrite by continuous IV infusion to achieve low nM (SNP) and 1uM (nitrite) levels in blood. The functional paradigm comprised 60 epochs of 10s off/5s on/15s off blocks of bilateral forepaw stimulation, with the stimulation parameters 2mA, 0.3-ms, 3 Hz.
Laser Doppler Flowmetry (LDF): a cranial window was installed over the somatosensory cortex of α-chloralose anesthetized Sprague-Dawley rats (n=5). An LDF monitor (Moor Instruments, Ltd, UK) was used to collect data from the constantly perfused chamber at the baseline, after systemic nNOS inhibition (see fMRI above for details) and after topical perfusion with SNP and nitrite (local concentration 10nM for SNP and 1μM for nitrite). Stimulation blocks consisted of 10s/5s/45s (off/on/off) unilateral forepaw stimulation, with stimulation parameters 2mA/0.3ms/3Hz

RESULTS:

Inhibition of nNOS decreased both the CBF and the BOLD fMRI responses ($p<0.05$), without affecting the resting CBF baseline, as previously reported [2] and as shown in Fig. 1A. In addition, nNOS inhibition also attenuated the LDF functional response ($p<0.05$), as shown in Fig 1B. Administration of the NO-donor SNP significantly recovered the BOLD fMRI response ($p<0.05$, Fig 1A), but not the CBF fMRI response ($p >0.05$). When evaluated by LDF, SNP significantly recovered the functional response ($p<0.05$, Fig. 1B). Interestingly, nitrite fully restored the neurovascular coupling to baseline levels (Fig 1B).

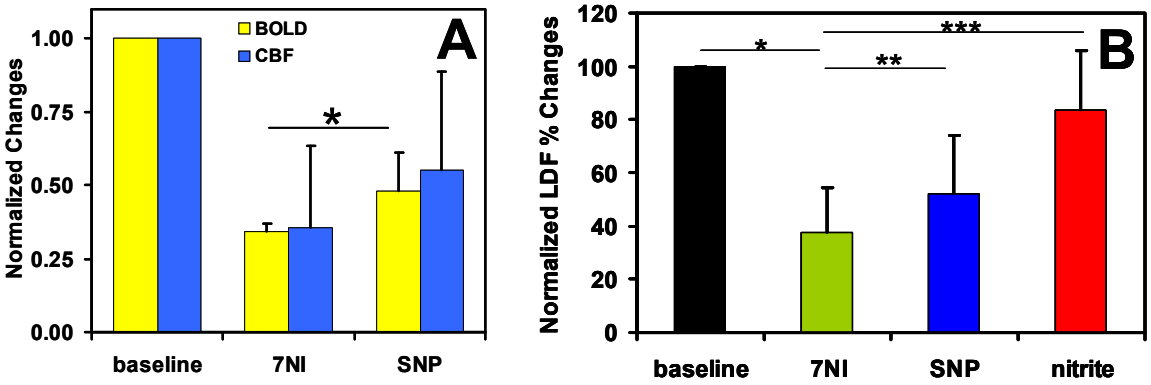

Figure 1. A: Normalized BOLD and fCBF after 7NI and SNP administration.
B: Normalized functional LD flow after 7NI, SNP and nitrite administration.

CONCLUSIONS:

Our present study brings further experimental evidence for the importance of NO as a mediator of neurovascular coupling. Comparison of the effect of direct NO donor SNP with that of NO precursor nitrite leads to hypothesis about different mechanisms in both cases, as nitrite is known to cause vasodilation not only by direct NO-releasing mechanism from its reaction with deoxyhemoglobin, but also by stimulating ATP release from erythrocytes and H_2O_2- related pathway in its reaction with oxyhemoglobin. All these pathways could be important in neurovascular coupling and especially for brain hemodynamic processes in cases of diseases and disorders related to impaired NO metabolism.

REFERENCES:

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