A Chemical Characterisation Of Hydrogen Sulfide-Mediated Production Of Nitric Oxide From Nitrite And S-nitrosoalbumin

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Until recently nitrite (NO_2^-) has been considered just an inert metabolite of nitric oxide (·NO). In fact it is an important biological reservoir of ·NO. NO_2^- alters hypoxic signalling and vasodilation, and protects the vasculature after ischaemia-reperfusion injury.

S-nitrosothiols (RSNOs), another example of ·NO-derived species, have similarly gained great attention for their vasodilatory properties and vascular protective effects after ischaemic events. The mechanism suggested to explain both NO_2^-- and RSNO-mediated effects is ·NO production. NO_2^- can be reduced to ·NO at low pH and low O_2 tension by xanthine oxidase, deoxyhaemoglobin, mitochondrial complexes and ascorbate.

Hydrogen sulfide (H_2S) is a recently discovered gasotransmitter which exerts many physiological effects similar to those of ·NO (e.g. vasodilation, promotion or inhibition of inflammation etc…)

The aim of this work was to test whether H_2S could promote ·NO production from NO_2^- and RSNOs. We also examined whether H_2S was able to interact with NO_2^- and RSNOs to affect cGMP production from human aortic smooth muscle cells (HASMC) and protect endothelial cells from oxidative stress-induced cytotoxicity.

·NO production from NO_2^- and S-nitrosoalbumin (SNOA) was chemically characterised by electron paramagnetic resonance (EPR) spin trapping and the spin trap used for the experiments was the Fe^{2+} complex of N-methyl-D-glucamine dithiocarbamate (MGD). ·NO was also assessed by gas phase ozone-based chemiluminescence. In the EPR experiments H_2S was generated using the sulfide salt NaSH. On addition of H_2S to NO_2^-, in the presence of the spin trap, a triplet EPR signal attributable to the NO-Fe^{2+}-$(MGD)_2$ spin adduct was detected. Peak integration of the EPR spectra of spin-trapped ·NO showed that 5 min incubation (at room temperature) of 1 mM NO_2^- with 1 mM NaSH increased ·NO formation compared to NO_2^- alone, with increases of 25.8±7.7% (mean± SEM) and 22.7±7.0% at pH 4 and pH 7.4, respectively (n=4) (final ·NO concentrations in the presence of NaSH were 579 ± 67 µM and 6.5 ± 1.9 µM at pH 4 and 7.4 respectively) . Chemiluminescence confirmed that H_2S rapidly reduced NO_2^- directly to ·NO, at pH 7.4 and that the reaction was thiol-dependent. Chemiluminescence also showed that, in the presence of NaSH, RSNO levels in SNOA significantly decreased (n=3, $p<0.01$, data analysed by 2-way ANOVA) almost as soon as the reagents were mixed together.

This work sets the basis to assess whether H_2S mediated-reduction of NO_2^- and RSNOs can be considered a physiologically relevant alternative pathway for ·NO production.