Hydrogen sulfide: a neurotransmitter or just a cofactor of the nitrite in the NO production?

by Loris Grossi

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Abstract.

The hydrogen sulfide has been recently claimed to have an important role in the cardiovascular system, as well in the central nervous system, but its action seems directly connected to the presence of NO/NO-derivatives. We want to report here chemical evidences that suggest for the H_2S a role as a cofactor, able to induce NO release from NO-donors, more than a direct neurotransmitter.

In the last decade great attention has been devoted to the role of the hydrogen sulfide (H_2S), *in vivo*, as a possible neurotransmitter,[1,2] and how it affects cardiovascular functions.[3,4] In the light of these stated roles, nowadays, particular attention is devoted to the possible synergy between H_2S and NO; for example, the positive role of H_2S in comparison to NO-releasers, i.e., increase of the NO production,[5] or the action of NO in inducing an increase of the amount of enzymes responsible of H_2S production. Thus, in several physiological processes the direct interaction between H_2S and NO is claimed, and hypothesized to lead to the formation of an intermediate S-nitrosothiol, even if not yet identified. To support this, results obtained in the incubation of the hydrogen sulfide donor, the sodium hydrosulfide (NaSH), with a range of NO-donors, or the direct interaction between NO gas and H_2S, are invoked; in particular, the reaction between the sodium nitroprusside (SNP) and the NaSH has been carefully investigated.[6]
But, an EPR study on the interaction between thiol derivatives and the sodium nitroprusside had already been conducted in our laboratory, and it led to conclude that an Electron Transfer process, induced by the thiol group, is involved.[7] It was also proved that the corresponding S-nitrosthiol is formed, but no reactants are required to accomplish the NO release from this intermediate.

Due to the net discrepancy between our results and those reported in the literature, sharper proofs to confirm that is the most reliable mechanism involved in this type of reaction were necessary. To this aim, the reaction between the SNP and the sodium hydrosulfide, in thoroughly deoxygenated water as solvent, was studied. Experiments conducted in EPR-sample tubes directly in the spectrometer cavity, let to detect only one paramagnetic species that was clearly identify as the reduced SNP radical,

$[Fe^{II}(CN)_5(NO)]^{3-}$ a_N = 1.49 mT and g = 2.0255, Figure 1a. To account for the formation of this unique species, an Electron Transfer process induced by the hydrosulfide anion (SH^-) could be hypothesized, and that supported by the pH value of the aqueous medium. In fact, because of the hydrolysis of the NaHS, the pH raises to *ca.* 8/9 favoring the presence of this anion. However, at pH around physiological, the sulfidric acid equilibrium moves towards the *aci*-form, H_2S, becoming preponderant, and maybe the possibly reducing species. To prove this, experiments were conducted in buffer solution at both pH 7.4 and pH 6.15: the $[Fe^{II}(CN)_5(NO)]^{3-}$ radical was still immediately detectable, confirming the capability also of the hydrogen sulfide to act as a reducing agent, Scheme (a).

In general, our evidences resulted to be in net contrast with those reported in the literature; for instance, the detection of only one and persistent radical species, and no intervention of any extra reagent, for any purpose, was contrasting with the use of $HgCl_2$,[6,8] an oxidant, claimed to be compulsory for inducing both the release of NO, from a hypothesized S-nitrosothiol intermediate, and to generate the reduced SNP radical. However, of these statements, the formation of the S-nitrosothiol intermediate, even if no account has been reported, could be reliable; in fact, an analogous behavior was evidenced in reacting glutathione with SNP, and the formation of the corresponding S-nitrosothiols by UV spectroscopy was detected.[7] But, a very weak S-N bond, which can undergo a rapid, spontaneous, homolytic cleavage, characterizes S-nitrosothiols, and in this experiment the hypothesized nitrosothiol intermediate would result the HS-NO, definitely unstable, which spontaneously releases NO without need any redox process.[9,10] So, it is very difficult to account for the involvement of $HgCl_2$ in such a process, and even more in the generation of the $[Fe^{II}(CN)_5(NO)]^{3-}$ radical. However, the most evident inaccuracy reported is the claim of the direct detection of NO radical by EPR spectroscopy:[6] it is well know the impossibility to conduct experiments with such a goal, and that for technical reasons.[11]

The experiment stated before stressed the role of the hydrogen sulfide in inducing the NO release from an exogenous NO-releaser, but the key aspect to be clarified was the reactivity of H_2S in comparison to NO and/or NO-releasers be present *in vivo*. In this light, we thought that the nitrite ion, the main pool of NO *in vivo*,[12] could be the most

important species involved, and then worth to be investigated. In fact, the claim of the direct interaction between NO and H_2S is in net contrast with the NO-chemistry, which let it just to react with radical species or coordinate to metal ions, and therefore, the results stated in the literature[6] could be eventually accounted for as the interaction between H_2S and NO-derivatives; for instance, oxidized species such as HNO_2 and/or N_2O_3.

To prove the possibly behavior of H_2S *in vivo*, i.e., the reaction with the nitrite to form NO, experiments with equimolar amounts of NaHS and $NaNO_2$, in carefully deoxygenated water, were carried out. In particular, the reaction was conducted under continuous bubbling of the solution with N_2 gas, and that to avoid a possible air-oxygen contamination, but also to remove and convey the likely NO formed into a deoxygenated methylene chloride solution of iron diethyldithiocarbamate, $Fe(DETC)_2$; the latter, an efficient NO trap, should lead to the formation of a paramagnetic species easily identifiable by EPR spectroscopy, Scheme (b). The mixture was then let to react for one hour, and the trap-solution analyzed by EPR: but no paramagnetic species could be detected. This result seemed to depone against our hypothesis, but we were aware that the nitrite reduction goes through its *aci*-form, i.e., HNO_2, whose concentration is regulated by the acid equilibrium constant and therefore by the pH of the medium.[13,14] Actually, in this experiment, the hydrolysis of both reagents, NaSH and $NaNO_2$, induces the increase of the pH up to *ca*. 10, and then a very low concentration of HNO_2 results available. Thus, pH values closer to the physiological, around 7, favoring higher HNO_2 concentrations, would necessary for verifying the hypothesis. Experiments were then repeated in buffer solution at both pH 7.0 and 6.15. When the $Fe(DETC)_2$ methylene chloride solution was analyzed by EPR, a paramagnetic species, whose spectroscopic parameters let it clearly identify as the NO adduct to the iron diethyldithiocarbamate, NO-$Fe(DETC)_2$, a_N = 1.28 mT g = 2.039, was detected, Figure 1b. The mixture was then let to react for 48 hours more and, from the aqueous solution, a yellow precipitate was recovered: it was identified by mass spectrometry as elementary sulfur, S_8. These results definitely proved the reducing capability of the HS^-/H_2S in comparison to the HNO_2, inducing NO release at pH around physiological.

In the light of these evidences, the possibility for H_2S to act directly as a neurotransmitter

seems not straightforward, but its action seems to depend, positively or negatively, on the interaction with an NO-releaser. Furthermore, the statements invoked to account for H_2S as a neurotransmitter are usually based on ending observations, i.e., cause-and-effect, and no mechanism has ever been reported, i.e., the chemistry of the interaction between H_2S and NO-derivatives has never been taken into account. For example, it is well acknowledge the role of H_2S in some vascular diseases such as hypertension, and its cardioprotective effects in ischemic myocardium or in septic and endotoxin shock,[15] but all these pathologies, examined from a chemical point of view, result to be characterized by the same ending: the increase of the blood acidity. Thus, the drop of the pH at value lower than the physiological could make the right conditions for the H_2S to act as reducing agent in comparison to the nitrite, whose acid equilibrium is now strongly shifted towards the *aci*-form, HNO_2, and then allowing the release of NO.

In definitive, it seems really risky to invoke the H_2S as a direct gas-transmitter without take into account the chemical conditions in which the bio-chemical process occurs; on the contrary, it seems more conceivable to consider H_2S just a cofactor of NO-releasers, most probably the nitrite *in vivo*, for inducing free nitric oxide: the proved neurotransmitter.

Acknowledgments.

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Figure and Scheme legends

Figure

EPR spectra. (**a**) Radical deriving from the SPN reduction induced by NaHS in aqueous solution at different pH. (**b**) Paramagnetic NO adduct to the $Fe(DETC)_2$; NO is formed in the reaction between $NaNO_2$ and NaHS, in aqueous solution, at pH ≤ physiological.

Scheme

Reaction mechanisms. (a) Reduction of SNP induced by the HS^- and/or H_2S, independently of the pH. (b) The pH-dependent reduction of the nitrite by the hydrogen sulfide; the formation of the labile S-nitrothiol intermediate followed by the trapping of the spontaneous released NO.

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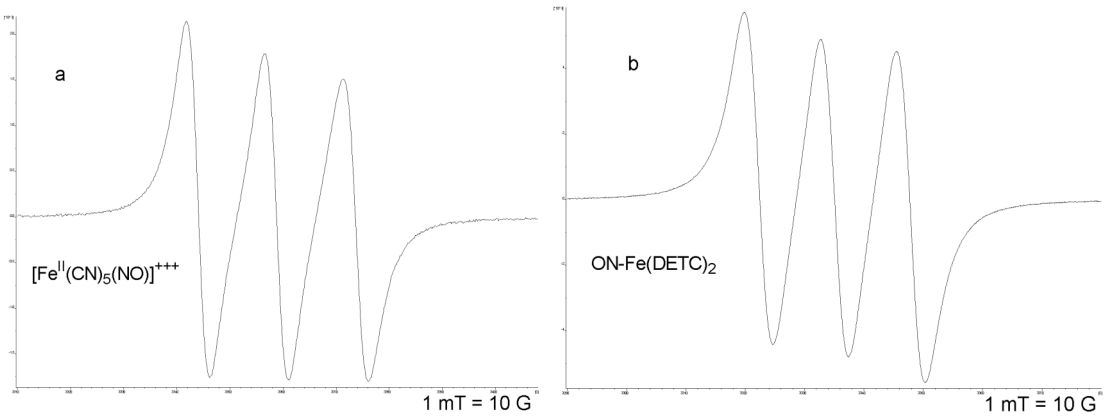
Figure



Scheme

a)

$$[Fe^{III}(CN)_5(NO)]^{2-} \xrightarrow{+ (HS^- / H_2S)} [Fe^{II}(CN)_5(NO)]^{3-}$$

Paramagnetic species

b)

$$HNO_2 \xrightarrow[pH\ \sim 7.0]{+ (HS^- / H_2S)} HS\text{-}NO \xrightarrow{\Delta} NO$$

$$Fe^{II}(DETC)_2 + NO \longrightarrow ON{-}Fe^{II}(DETC)_2$$

Paramagnetic species

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Graphycal Abstract