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Review

Is exogenous hydrogen sulfide a relevant tool to address physiological questions on hydrogen sulfide?

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ABSTRACT

This review challenges the use of solutions of dissolved exogenous H_2S in the literature as a tool to determine the potential physiological functions of endogenous H_2S as well as its putative therapeutic applications.

Our major point of contention is that solutions of dissolved H_2S are used in vitro at concentrations, within the high microM range, which are above the concentrations of dissolved H_2S found in blood and tissues during lethal H_2S exposure in vivo. In addition, since the levels of toxicity are extremely variable among cell types, a property that is seldom acknowledged, the physiological relevance of data obtained after local or in-vitro administrations of H_2S at concentrations of few microM is far from certain. Conversely, the rate of disappearance of the dissolved pool of H_2S in the body (being trapped or oxidized), which we found to be at least of several micromoles/kg/min, is so rapid in vivo that if relatively low *quantities* of H_2S, i.e. few micromoles for instance, are administered, no change in H_2S concentrations in the body is to be expected, unless toxic levels are used. Protocols looking at the effects of compounds slowly releasing H_2S must also resolve a similar conundrum, as their effects must be reconciled with the unique ability of the blood and tissues to get rid of H_2S and the steepness of the dose-toxic effects relationship.

Only by developing a comprehensive framework in which H_2S metabolism and toxicity will be used as a rationale to justify any experimental approach will we be able to bring definitive evidence supporting a protective role for exogenous H_2S, if any, and its putative function as an endogenous mediator.

Contents

What effects can we expect to be produced by a 5 μL solution containing dissolved hydrogen sulfide (H_2S/HS^-) at the concentration of 2.5 *mM* directly administered into the cerebro-ventricles of a rat? It is, in substance, one of the fundamental questions raised by Li et al. (2016) in their recent study published in this journal.

The approach consisting in injecting high microM or even milliM solutions of dissolved H_2S directly into an organ or in the milieu bathing cells in culture has been repeatedly used in the literature [see for list of references (Nicholson and Calvert, 2010; Szabo, 2007; Szabo et al., 2011)] with 2 main objectives: (1) determine whether exogenous H_2S possesses therapeutic properties, such as for instance limiting the consequences of an hypoxic/anoxic insult or (2) speculate about the possible role of "endogenous" H_2S or its products of oxidation.

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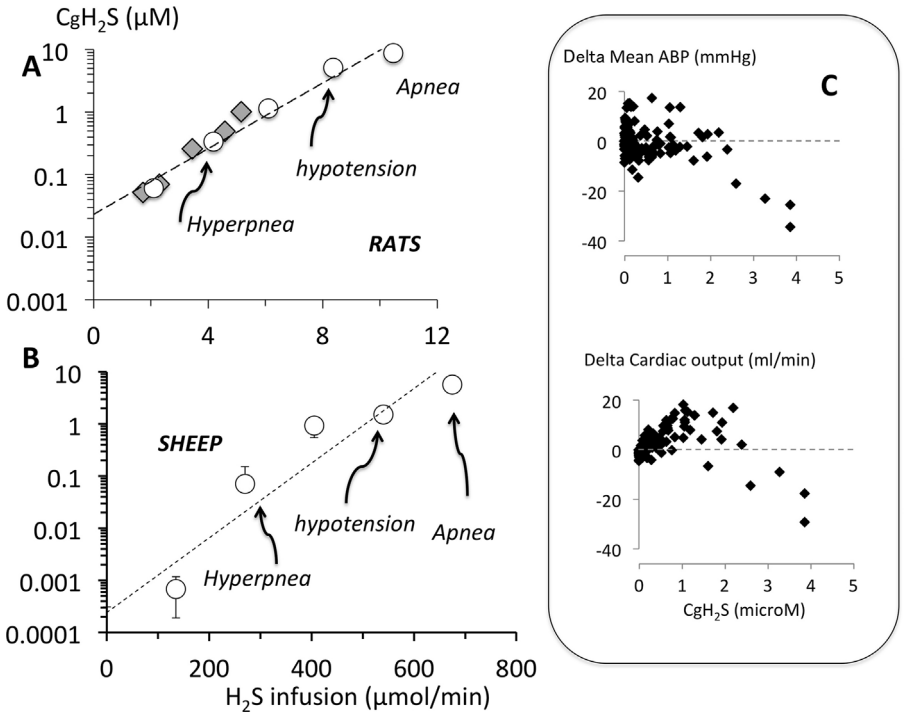

Fig. 1. A: Concentration of gaseous H_2S in the arterial blood (CgH_2S) estimated from alveolar H_2S as a function of the rate of H_2S infused (only mean values are shown) in a group of rats. Breathing was stimulated at concentrations of gaseous H_2S ranging between 0.34 and 1.14 µM while the highest values corresponding to the lethal level ranged from 5.09 to 8.80 µM. The data recomputed from the study of Insko et al. (2009) (diamonds) fit with the relationship established in the present study. Modified from Klingerman et al. (2013). Of note these concentrations represent 1/3 of total Free H_2S, i.e. H_2S/HS^-, present in the blood. B: Concentration of gaseous H_2S in the arterial blood (CgH_2S) estimated from alveolar H_2S as a function of the rate of H_2S infused in a group of sheep. Breathing stimulation and apnea were produced at levels similar to those found in the rats. Modified from Haouzi et al. (2014) C: Individual data points obtained in a group of rats during infusion of NaHS. Note that when CgH_2S reached concentrations of about 2–3 µM (less than 10 µM total free sulfide), a progressive drop in blood pressure associated with a reduction in cardiac output was observed. Note the very narrow range of H_2S between trivial and life-threatening effects. Modified from Sonobe and Haouzi (2016).

For all intents and purposes, the argument presented in this viewpoint is that solutions of exogenous H_2S above a few microM, when in direct contact with cells or tissues produce effects that are toxic or even lethal in vivo (cardiac arrest, coma with neuronal necrosis). In contrast, cells or tissues containing large amount of metallo-compounds or expressing high level of sulfide quinone reductase activity, e.g. colonocytes or heptacoytes, are able to trap or oxidize very large amount of H_2S. These specific cells are immune to both the toxicity as well as "physiological" effects produced by sulfide in other cells or tissues. Conversely, low doses injected in vivo are probably not able to increase H_2S in a significant manner, since the blood (hemoglobin and proteins) can prevent any free sulfide to diffuse in a measurable manner into the tissues, unless toxic levels are used. Perhaps more importantly, due to the multifarious effects of H_2S, it is, for now, very difficult to define a specific function for H_2S in vivo based on any given defined H_2S-target interaction described from in vitro experiments.

1. What concentration of H_2S is toxic?

Solutions containing H_2S, prepared from NaSH or Na_2S, have been used as a source of sulfide to test the effects of hydrogen sulfide in various in vitro or in vivo studies [see for general review and discussion (DeLeon et al., 2012; Furne et al., 2008; Levitt et al., 2011; Olson, 2012, 2011)]. The sulfides dissolved in these solutions are, at a physiological pH of about 7, composed in large part of HS^- in equilibrium with a smaller portion of dissolved/free gaseous H_2S (Almgren et al., 1976; Carroll and Mather, 1989; Douabul and Riley, 1979). When used in cell cultures or isolated tissues, as well as when directly injected into an organ, the amount of H_2S able to diffuse into cells is therefore proportional to the partial pressure of the gaseous form of H_2S and thus the concentration of total free/dissolved H_2S/HS^-. Impurities and products of sulfide oxidation may be present in solution, their roles remain to be clarified (Nagy, 2015). Of note, when prepared without agitation and immediately before the experiment using a sealed container, concentration of gaseous H_2S remains relatively stable over time (at least for one hour) (Van de Louw and Haouzi, 2013). As soon as the solution of H_2S is added into a dish, evaporation of gaseous H_2S takes place and the concentration of soluble H_2S/HS^- exposed to the cells or tissue decreases in the milieu depending on many factors including the temperature, the pH, the surface of exchange and the level of agitation. In most experimental conditions, if the solution is neither agitated nor ventilated, a concentration in high microM or milliM range is going to remain present within the same order of magnitude for many minutes, a time long enough to produce a lethal effect (Judenherc-Haouzi et al., 2016). Many of these points have been discussed in details in recent reviews (Olson, 2012; Olson et al., 2014).

It should be kept in mind that H_2S is one of the most toxic mitochondrial poisons, even more toxic than cyanide on a mole-to-mole basis. In vitro, the activity of the mitochondrial cytochrome *c* oxidase is abolished by a solution of H_2S at concentrations of H_2S/HS^- ranging from 10 to 30 µM (Cooper and Brown, 2008; Leschelle et al., 2005; Yong and Searcy, 2001). This effect appears however to develop at much lower concentrations in certain tissues (neurons), while cells like the colonocytes can survive exposure to milliM concentrations of free H_2S for long period of time. In vivo, a depression in respiratory medullary neurons (leading to a fatal apnea within minutes) and a severe depression in cardiac contractility (leading to a terminal asystole within seconds) can be produced in rodents and in large mammals (Fig. 1) by infusing or inhaling H_2S at levels yielding blood concentrations of gaseous H_2S between 2–5 µM (Haouzi et al., 2014; Klingerman et al., 2013; Sonobe and Haouzi, 2016),