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Review

Controversies and conundrums in hydrogen sulfide biology

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ABSTRACT

Hydrogen sulfide (H_2S) signaling has been implicated in physiological processes in practically all organ systems studied to date. At times the excitement of this new field has outpaced the technical expertise or practical knowledge with which to accurately assess these advancements. Recently, the myriad of proposed H_2S actions has spawned interest in using indicators of H_2S metabolism, especially plasma H_2S concentrations, as a means of identifying a variety of pathophysiological conditions or to predict clinical outcomes. While this is a noteworthy endeavor, there are a number of contraindications to this practice at this time. First, there is little consensus regarding normal, i.e., "physiological" concentrations of H_2S in either plasma or tissue. In fact, it has been shown that the methods most often employed for these measurements are associated with substantial artifact. Second, interactions, or presumed lack thereof, of H_2S with other biomolecules (e.g., O_2, H_2O_2, pH, etc.) or analytical reagents (e.g., reducing reagents, *N*-ethylmaleimide, phenylarsine, etc.) are often assumed but not evaluated. Third, the experimental design and/or statistical analyses may not be sufficient to justify using H_2S concentration in tissue or blood as a predictive biomarker of pathophysiology. In this study, we first briefly review the problems associated with plasma and tissue H_2S measurements and the associated errors and we provide some simple methods to evaluate whether the data obtained is physiologically relevant. Second we provide a brief analysis of H_2S interactions with the above biomolecules. Third, we provide a statistical tool with which to determine the clinical applicability of H_2S measurements. It is hoped that these points will provide a rational background for future work.

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1. Introduction

The idea that nitric oxide (NO), heretofore only known as a toxic gas, could serve as a signaling molecule began in the late 1970s but it took over 10 years before it was embraced by the general scientific community [1]. Carbon monoxide (CO) didn't fare much better, even though its toxic characteristics had been extensively studied, and another 10 or so years passed between its first demonstration as a signaling molecule and the general appreciation of its biological significance. The biology of hydrogen sulfide (H_2S) has had a different chronology. Following the initial observations by Kimura's group that hydrogen sulfide (H_2S) elicited physiological responses in neuronal and cardiovascular tissues [2,3] it took only 2 years for these findings to be appreciated and an exponential rise in H_2S-related publications ensued. This is understandable as many careers were founded on NO and CO biology. However, in all this exuberance there has been less of a tendency for introspection and critical analysis. A number of reviews have been published recently that specifically address some of the misinformation and more obvious errors in this field [4–11]. Yet even today these cautions are often ignored and some misconceptions and errors have become more or less firmly entrenched. Now that there is heightened interest in the application of H_2S biology in the clinical setting, in both diagnosis and potential treatment, it becomes more imperative to correct these errors. The purpose of this review is to once again elaborate on the pitfalls (controversies) and some of the unsolved problems (conundrums) in this field. We also provide an easy-to-use statistical tool with which to evaluate the validity of clinical predictions, assuming this is accompanied by advances in plasma and tissue H_2S measurement. The hope is that this review will allow investigators to critically evaluate the literature as well as to more appropriately design their own experiments.

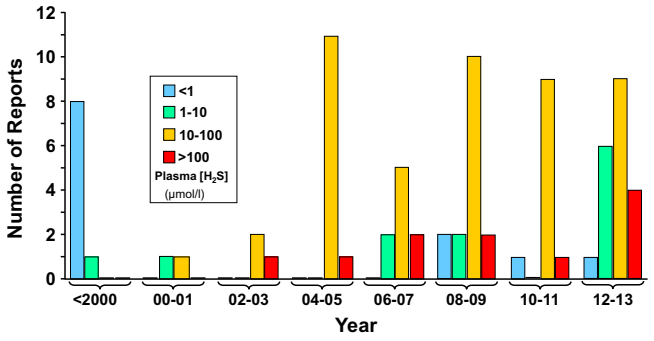

Fig. 1. Frequency distribution of the number of publications that have reported plasma or blood sulfide concentrations in the ranges indicated in the inset. Note the essential absence of H_2S in blood prior to 2000 and the current trend for increasing plasma H_2S concentration over the past 10 years. Modified from [7], with permission.

2. Blood and tissue H_2S concentration: methods and values

Perhaps the most controversial issues, and in our opinion must be the first to be corrected, are the methods used to measure H_2S and an appreciation of the values obtained from these measurements. As shown in Fig. 1, prior to 2000, and for all practical purposes before Kimura's papers [2,3] were promulgated, essentially all measurements of blood H_2S either failed to find any detectable H_2S, or, if found, the levels were extremely low. However, since 2000, the "average" concentration of H_2S in blood has steadily increased and by 2012–2013 this "average" has risen to nearly 50 μmoles/l in control animals (although H_2S as high as 600 μM has been reported in asthmatic patients [12]). Why this cannot be requires evaluation of the methods used and we offer a few simple "self-check" tools that can alert the investigator to potential errors. These are described in the following sections.

2.1. Methods

The most commonly employed methods for measuring H_2S concentration in blood or plasma are the methylene blue method (MB), sulfite-sensitive ion selective electrodes (ISE), monobromobimane (MBB), HPLC or GC analysis of headspace gas (HG) and amperometric (polarographic) electrodes. While, many of these methods are suitable for H_2S measurement in water or buffers they become less reliable in blood and tissue. Many of these assays are performed under anoxic or very hypoxic conditions and because this can affect the normal balance between H_2S production and oxidative metabolism [13] H_2S values can become spuriously elevated. The use of each method and the problems associated with their use are described in the following sections. Additional details can be found in recent reviews [4,7,14,15].