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Developing effective countermeasures against acute hydrogen sulfide intoxication: challenges and limitations

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Abstract

Hydrogen sulfide (H₂S) is a chemical hazard in the gas and farming industry. As it is easy to manufacture from common chemicals, it has also become a method of suicide. H₂S exerts its toxicity through its high affinity with metallo-proteins, such as cytochrome *c* oxidase and possibly via its interactions with cysteine residues of various proteins. The latter was recently proposed to acutely alter ion channels with critical implications for cardiac and brain functions. Indeed, during severe H₂S intoxication, a coma, associated with a reduction in cardiac contractility, develops within minutes or even seconds leading to death by complete electro-mechanical dissociation of the heart. In addition, long-term neurological deficits can develop due to the direct toxicity of H₂S on neurons combined with the consequences of a prolonged apnea and circulatory failure. Here, we review the challenges impeding efforts to offer an effective treatment against H₂S intoxication using agents that trap free H₂S, and present novel pharmacological approaches aimed at correcting some of the most negative consequences of H₂S intoxication.

Keywords

H₂S; pulseless electrical activity; apnea; hydroxocobalamin; methemoglobin; methylene blue; hydrogen sulfide

Introduction

Hydrogen sulfide (H₂S) remains a significant chemical hazard ^{1, 2} in gas and oil industry ^{3–6}, and in various farming ⁷ as well as fishing activities ⁸. It is also used as a method of suicide ⁹. This form of suicide ¹⁰ is accomplished by mixing, in a closed environment, a source of sulfide and various types of acidic solutions, which are readily available in most household chemicals ^{11, 12}.

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Conflicts of interest

The authors declare no conflicts of interest.

The consequences of an acute exposure to H₂S are usually benign as long as the level of exposure remains in the low ppm ranges, but as soon as exposure reaches levels above 500 ppm, the toxicity of H₂S towards the heart and the central nervous system can lead to rapid death or produce dreadful long-term neurological deficits⁴. Acute toxicity in surviving patients is associated with lesions of the olfactory epithelium,¹³ as well as pulmonary lesions,¹⁴ leading to a clinical picture of acute respiratory failure often requiring mechanical ventilation.

In this short review we will present some of the specificities of H₂S poisoning that, in our view, represent a major challenge when developing antidotes or countermeasures. Indeed, one of the major factor impeding our effort to come up with an effective treatment against H₂S intoxication *after* exposure is that the pool of free/soluble H₂S disappears from the body as soon as the exposure ceases^{15, 16}, preventing agents trapping free H₂S (cobalt or ferric compounds) to play their protective role. Yet, H₂S still exerts its toxicity well beyond the phase of exposure through mechanisms, which are not yet fully understood, but are not related to the persistence of free H₂S^{15, 16}. After a very brief presentation of our current understanding of H₂S metabolism, we will discuss some of the most recent results on the efficacy and limits of trapping agents and propose a new paradigm to treat some of the most dramatic complications of acute H₂S intoxication.

Acute H₂S intoxication: short- and long-term effects

H₂S toxicity–dose relationship is extremely steep and life-threatening symptoms can be produced by inhaling no more than 0.07% H₂S for as little as a few minutes. Figure 1 and 2 illustrates how relatively small increases in the level of exposure to infused H₂S can advance symptoms from a stimulation of breathing, to an apnea, and finally to a very rapid cardiac arrest, for blood concentrations of H₂S of a similar order of magnitude (Fig. 2).

During intoxication in humans and in un-sedated animals, acute exposure to H₂S provokes a rapid coma^{3, 17}. H₂S-induced coma is often referred to as *knock-down*, a term coined to illustrate the spontaneous and rapid reversibility of the coma if the exposure is of short duration. Knock-down is, however, far from benign, as it can be fatal¹⁸ within minutes whenever concomitant acute cardiogenic shock develops¹⁹. Our experience in rats and sheep exposed to toxic levels of H₂S shows there is a very fine line between a benign form of intoxication and a lethal one.

Death due to H₂S poisoning is secondary to a pulseless electrical activity (PEA) i.e. cardiac contractions are abolished while rhythmic electrical activity persists^{19–22}, which can very rapidly develop, as an endpoint of a primary depression in cardiac contractility. We found that ventricular fibrillation occurs more rarely than PEA. We also found that an isolated apnea, i.e. without any primary cardiac failure, can only be observed in a very narrow range of H₂S exposures, which can be uniquely created in the artificial setting of an experimental protocol. Indeed, although apnea induced anoxia can precipitate the development of a PEA and may contribute to brain ischemic/hypoxic injury²³, the blood concentrations of H₂S able to reduce breathing already provokes a significant alteration in the cardiac function^{15–62} (Fig. 2). Consequently, for all intents and purposes the presence of a depression in breathing

should always be seen as a marker of a severe primary depression in cardiac function. The basis for these contentions was described in the following studies^{18–22}. Note that in all the following experiments, H₂S was prepared from NaHS, dissolved in saline. The term “H₂S solution” will be used instead of “NaHS” throughout the manuscript for clarity. In one of these studies, using the relationship we previously established between H₂S concentrations and circulation²⁰, we studied the effects of H₂S at a rate of infusion (10 µM/min) that would be lethal in 10 minutes in rats ($n = 14$) anesthetized by urethane (1.2 mg/kg, IP) and mechanically ventilated. Arterial blood pressure and left ventricular pressures were monitored using PE-50 catheters. Transonic flow probes (MA2PSB) were placed around the ascending aorta, following median sternotomy, and around one femoral artery to measure cardiac output and femoral blood flow respectively (Fig. 4). We found that H₂S dramatically depresses cardiac systolic function, within less than one minute of exposure, resulting in a cardiogenic shock leading to PEA within 5 to 7 minutes (Fig. 4). Similar cardiac effects were found in a study involving sixteen sheep²². Of note, PEA can develop even after the end of exposure (Fig. 5). In non-sedated animals that are breathing spontaneously, minute ventilation is also depressed through a centrally inhibition of the medullary respiratory neurons, but the period of apnea is replaced by a gasping pattern¹⁸ that can be maintained for long periods of time. This gasping pattern allows auto-resuscitation to occur, if circulation is maintained or restored¹⁸. As mentioned earlier, in less dramatic intoxications, the clinical manifestations consist in a syncopal episode and a persistent period of hypotension²³. Long-term cognitive or motor deficits can still develop. These Deficits have been shown to take place in animal models¹⁸ and in humans²⁴. We found that in a model of intoxication leading to a 75% mortality, 25% of surviving animals had neurological lesions¹⁸, which included diffuse neuronal necrosis of the cortex, putamen, and thalamus. The hippocampus and the cerebellum were spared, in contrast to what is typically seen in post-anoxic injury. This description suggests that although neuronal necrosis could be facilitated by the ischemic insult secondary to severe circulatory depression and hypoventilation induced hypoxemia²³, H₂S seems to produce genuine neuronal lesions, different from those produced by anoxia¹⁸. Finally, acute lesions of the olfactory epithelium^{13, 25} and the lungs²⁶ are present during a single exposure to inhaled H₂S starting from 400 ppm.

The mechanisms of H₂S toxicity remain debated

The toxicity of H₂S has long been considered to result from its combination with the ferric heme a₃ of the mitochondrial cytochrome *c* oxidase²⁷, inhibiting its activity^{28, 29}, in turn preventing ATP formation and promoting the production of reactive oxygen species (ROS)³⁰. However, recent works on the interaction between H₂S and proteins³¹, including ion channels³², suggest that H₂S could affect cysteine residues of most proteins by direct “sulfhydration” or “sulfuration” of free cysteine residues (S-SH bonds)³³. This mechanism has been put forward to account for the extreme and early cardiac toxicity of H₂S on Ca²⁺ channels^{32, 34}, including on the sarcoplasmic reticulum ryanodine receptors (RyR) rich in free cysteine residues. Due to the multitude of metallo-proteins physiologically present in the cells and in the blood (from hemoglobin in the blood to myoglobin in the heart and muscles) and the high rate of H₂S oxidation, it is unclear if and how much free hydrogen

sulfide could combine to cysteine residues of proteins in vivo. Alternatively, an increased ROS production—a result of the impediment of the electron chain activity³⁵—can also affect the activity of the calcium channels³⁶. Regardless of the mechanisms involved, various ion channels, including K⁺-ATP³⁷ or Ca²⁺^{32, 34, 38} channels, can be affected by H₂S in the heart and the central nervous system through mechanisms of toxicity that do not always require the mitochondrial activity to be altered, as shown for the medullary neurons³⁹. A comprehensive description of the contribution of ion channels, “reconfiguration” of proteins, or mitochondrial dysfunction to the symptoms of sulfide poisoning is cruelly missing^{15, 16, 18, 19}. These effects persist for a much longer time than soluble H₂S in the body²² during severe intoxication and can explain the persistence of signs of toxicity even when all free H₂S has vanished.

Kinetics and metabolism of free H₂S, a challenge for the treatment of H₂S poisoning

H₂S, which is much more soluble than CO₂ or O₂^{40–42}, diffuses almost instantaneously into the blood as soon as it is inhaled and then to all tissues and cells. We have characterized the fate of the various pools of H₂S in the blood¹⁵, their kinetics as well as their links to the clinical manifestations and symptoms of toxicity^{15, 16}.

In the blood, only a very small portion of sulfide remains in a “free/soluble” or diffusible form, comprising the gaseous form H₂S^{41, 43} and the sulfhydryl anion HS[−].⁴⁴ A larger pool of sulfide will combine with metallo-proteins such as the ferrous iron of hemoglobin or may, as presented in the previous paragraph, react with cysteine residues present in a large number of proteins⁴⁵, creating a sink for H₂S. While free/soluble H₂S is the only form able to diffuse into the cells, “combined” H₂S represents the toxic pool.^{15, 16} A schematic representation of the fate of H₂S is shown in Figure S1. The most remarkable feature of H₂S metabolism is that H₂S disappears *spontaneously* from the blood and the tissue^{15, 46, 47} at a very rapid rate (Fig. S2). Indeed H₂S is almost immediately oxidized in the mitochondria, a reaction catalyzed by the mitochondrial sulfide quinone oxido-reductase (SQR).⁴⁸ Of note, the sulfur transferase enzyme, rhodanese, has been proposed to contribute to H₂S detoxification/elimination⁴⁹. Thiosulfate, resulting from spontaneous oxidation of H₂S, could, after reacting with one of the cysteine residues of rhodanese, create a persulfide. In the presence of cyanide, thiocyanide could then be formed. However, rhodanese, can only be involved *after* the conversion of H₂S/HS[−] into thiosulfate, eventually catalyzing the conversion of thiosulfate to thiocyanate. This enzyme does not metabolize sulfide per se. As demonstrated by Wilson *et al.*,⁵⁰ the rate-limiting step in H₂S detoxification is its oxidation to thiosulfate; the conversion of thiosulfate to thiocyanate by the rhodanese does not increase the rate of sulfide detoxification. H₂S also disappears spontaneously from a solution of proteins or from the blood, transformed into polysulfide, thiosulfate, sulfite, and sulfate in the presence of oxygen. We found that all these potential mechanisms of oxidation can account for the disappearance of soluble H₂S, which occurs within one minute in large and small mammals^{15, 16} following a period of exposure to sulfide (Fig. S2), while the “hidden” forms of combined sulfide can persist for longer periods of time.⁵¹ As a consequence, the toxic effects of H₂S persist beyond the phase of exposure without being accessible to an

antidote that would primarily act on the free/soluble form. Only in very severe exposure that could be very rapidly lethal would H₂S be unable to be oxidized at the moment of death, and thus could persist in large quantity in the body. During these extreme intoxications, no treatment could be administered on time, as death would be almost immediate. We found that in animal models, non-immediately lethal intoxications, even if severe, are associated with concentrations of H₂S in blood that would be at the most in the high micromolar range.¹⁵ At these levels, a total disappearance of H₂S is to be expected as soon as the patient is removed from the source of exposure.

Therefore, the challenge is to find compounds antagonizing the effects of H₂S toxicity while the pool of free or exchangeable sulfide is already gone. Yet, the treatment of H₂S poisoning has been traditionally aimed at trapping free H₂S using metallo-compounds, e.g. ferric iron contained in methemoglobin^{52–54} or cobalt in hydroxycobalamin (HyCo).^{54–56} Nitrite-induced methemoglobinemia^{57, 58} can, however, only trap H₂S outside the cells and have little or no effects on the combined forms, after exposure.¹⁶ In addition, sodium nitrite can decrease arterial blood pressure in subjects already in shock and affect oxygen transport. Cobalt contained in HyCo⁵⁴ has several theoretical advantages over methemoglobinemia.^{55, 59} Whether this very large molecule can penetrate cells at sufficient concentrations rapidly enough relies on the few data that have been gathered mostly for the study of cyanide poisoning⁶⁰ and thus remains to be convincingly demonstrated. We found that HyCo (70 mg/kg) reduces the immediate mortality of sulfide in the sheep, but only when used within one or two minutes following exposure.²² In our experience, no clear beneficial effects are demonstrable if HyCo is administered later on, and more importantly HyCo does not prevent the development of a severe metabolic acidosis or lactate production in the surviving animals.²² As HyCo has no interaction with sulfide “fixed” on proteins, new paradigms must be proposed using agents counteracting the deleterious consequences of H₂S toxicity, rather than trying to trap soluble H₂S.

Methylene blue: a new treatment of H₂S poisoning using an old molecule?

Methylene blue (MB) is a cationic tri-heterocyclic redox compound with a central aromatic thiazine ring system, for which pharmacokinetics, both in the blood and tissues, has been established in various species including humans^{61, 62}. MB is routinely used for the treatment of methemoglobinemia at the dose of 1–2 mg/kg IV⁶³. MB diffuses extremely rapidly and accumulates in all tissues including the heart and the brain⁶². MB has been shown to have a relatively long half-life in the blood (4–5 h in the rat) and is reduced to leucomethylene blue (LMB) in the blood and in the cells. LMB forms with MB a reversible oxidation-reduction system or electron donor-acceptor couple (see Refs. 64 and 65). In tissues, the main form of MB is LMB, which accounts for the potent reducing properties of MB. Inside cells, MB/LMB concentrates in mitochondria where it can interact with mitochondrial complexes. We have found that MB is a potent agent against H₂S poisoning, when injected after the exposure, changing the immediate prognosis of severe intoxication and improving the long-term neurological outcome^{18, 19}. We have established that this effect is primarily related to the restoration of cardiac function, preventing PEA and

restoring normal breathing^{18, 19}. In these experiments, rats ($n = 34$) received intraperitoneal (IP) injection of 20 mg/kg NaHS solution every 10 minutes until they presented a coma. On average, two injections were needed to produce a coma within 1–2 minutes; two minutes into the coma, defined as the loss of righting reflex, animals started gasping (Fig. S3). This phase led to spontaneous recovery or cardiac arrest within 7 minutes. Three minutes into the coma, the rats received either saline or MB (IV tail vein, 4 mg/kg $\times$ 2). Surviving rats underwent clinical examination, open field and Morris Water Maze (MWM) testing for 4 days starting 1 day after the intoxication. As shown in Figure S4 the immediate mortality was dramatically decreased after injection of MB (75% survival, $P < 0.01$). No rats were found with early neurological deficits in the MB treated group; furthermore, they displayed an effective spatial search strategy (direct, directed or focal) during MWM testing with a significantly higher incidence than in the control intoxicated animals (Fig. S4).

As a follow-up study and using a continuous infusion of NaHS (10–11 micromoles/min) in anesthetized rats that would be lethal in about 7–10 minutes¹⁹, MB (4 mg/kg) or saline was injected as soon as the cardiac output reached 40% of its baseline value (within 2–3 minutes). We found that MB injection restored cardiac contractility (Fig. 5) blood pressure and cardiac output with very fast kinetics, and significantly prolonged the time to death¹⁹. Finally, to establish whether MB could affect the concentrations of H₂S in solution during *in vitro* experiments, the amount of gaseous H₂S released from a solution prepared from NaHS (100 microM, final quantity 1.5 micromoles) was measured in saline or PBS with or without MB (20mg/l). Solutions were placed in a sealed vial and the amount of H₂S diffusing in the gaseous phase was analyzed after 10 minutes of incubation. We found that the entire amount of H₂S present in solution was recovered from the medium, with fast kinetics, regardless of the presence of MB. This confirms our previous data¹⁹ that no direct interaction between soluble H₂S and MB are present.

Potential mechanisms of action of Methylene blue

First, LMB has potent reducing properties. This effect⁶⁵ has been proposed to account in part for the restoration of cognitive deficit in various models of brain dysfunction⁶⁴ and in post-anoxic brain injury⁶⁶. It is possible that it is through its reducing properties that LMB could rapidly restore the normal configuration of proteins in Ca²⁺ channels in the heart by cleaving disulfide bonds newly formed by SH⁻ during sulfide intoxication^{32, 34}. The reducing properties of LMB could also affect the redox modulation of RyR or other calcium channels in relation to the numbers of sulfhydryl groups present. Indeed, H₂S can lead to the accumulation of reactive oxygen species via a decrease in the electron transport chain activity³⁶, a damage that can be opposed by MB/LMB properties. MB has also been shown to reduce post-anoxic brain injury produced by a cardiac arrest^{66, 67}, which could account for the improvement of the longterm neurological outcome that we found. Second, MB supports the transfer of protons through the mitochondrial membrane against a concentration gradient, essential for the production of adenosine triphosphate (ATP), when mitochondrial respiration is impeded^{68–70}. Although this property remains quite controversial, MB could antagonize the mitochondrial mechanisms of hydrogen sulfide toxicity^{29, 35}. This mechanism may also account for the remarkable protection by MB against the toxic effects of the acute or chronic exposure⁷¹ to sodium azide⁷², another mitochondrial poison. Third,

MB at low dose exerts a potent anti-nitric oxide (NO) effect ⁷³, as MB inhibits guanylyl cyclase. This anti-NO effect ⁷⁴ is the basis for treating refractory post-operative vasoplegia ^{75, 76}, and could also be of interest during H₂S poisoning.

Outstanding questions

How to treat intoxication by a gas, of which the pharmacokinetics and the mechanisms of toxicity remain poorly understood, is certainly a challenge. Trapping H₂S, using cobalt or ferric compounds, is extremely effective on the pool of free H₂S, but as this pool vanishes very rapidly after exposition, the relevance of these compounds on the intracellular pools of combined sulfide is problematic. Methylene blue does not combine with H₂S and does not appear to affect its metabolism, but it is able to counteract some of the very acute effects of H₂S on the heart and possibly the delayed effects of sulfide on the central nervous system. The acute effects of MB are of short duration following an IV administration but appears to be long enough to allow survival. It is unclear whether MB is effective during severe depression in cardiac contractility, wherein various and complex mechanisms of depression could be involved. Our present results suggest that the efficacy of MB decreases, as cardiac depression is more profound ^{18, 19}. The benefit of other phenothiazium chromophores ¹⁹, in association with “trapping” antidotes and specific cardiotoxic agents must be investigated, keeping in mind that any increase in O₂ demand may be deleterious for the heart and the central nervous system.

Conclusions

The effects of hydrogen sulfide (H₂S) intoxication have too often been reduced to those that this gas shares with cyanide: an inhibition of the mitochondrial cytochrome *c* oxidase activity. However, H₂S displays a unique fate, as its soluble/diffusible form disappears extremely rapidly from the blood and tissues. If H₂S does not kill a patient within seconds or minutes, it can dramatically affect cardiac contractility and affect various cortical and medullary functions via mechanisms not always related to a reduction in ATP production.

MB injection during the agonal phase of H₂S induced coma in the rat is able to increase the survival rate and to improve the neurological outcome. These effects are the result of the rapid restoration of cardiac contractility depressed by sulfide. Whether MB counteracts the effects of H₂S at the level of the Ca²⁺ channels or through another molecular mechanism remains to be determined. Finally, the doses at which MB should be used in a clinical setting await clarification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Recordings in three urethane anesthetized rats showing the effects on breathing (inspiratory flow, $\dot{V}$), blood pressure (ABP) and carotid blood flow (BF) of a bolus injection of a solution containing H_2S at three different concentrations. The first observable symptom in this model was produced at a concentration of about 3 $\mu\text{mol/kg}$ and consisted in a transient increase in breathing secondary to the stimulation of the arterial chemoreceptors. At 9 $\mu\text{mol/kg}$, animals would display a central apnea and a severe hypotension. In this example, death was prevented by initiating mechanical ventilation, as illustrated by the changes in airway pressure (airway P), allowing spontaneous breathing to resume and circulation to return to its baseline levels after several minutes. Doubling this dose provoked an immediate and irreversible shock leading to a rapid asystole, regardless of the ventilatory support. Modified from Ref. 77.

Figure 2.

Relationship between the estimated concentrations of gaseous H_2S (soluble) and the rate of H_2S infused in rats. CgH_2S refers to the concentration of gaseous form of H_2S , while CDH_2S is the form combining CgH_2S and the concentration in HS^- . CmmbH_2S represents the concentration the total pool of H_2S present in the blood determined by the monobromobimane (MBB) method. Modified from Refs. 15 and 16). Note that breathing was stimulated at concentrations of CgH_2S around or even below $1 \mu\text{M}$ in the blood, while lethal concentrations are about $8 \mu\text{M}$ only. Left panel shows the comparison between rats and large animals (sheep). Note that symptoms are produced in both species at similar levels of soluble gaseous in the blood. Sheep seem to have a higher pool of total H_2S than rats.

Figure 3.

Example of recordings obtained in anesthetized and mechanically ventilated rats, showing the effects of a continuous infusion NaHS (10 $\mu\text{mol}/\text{min}$) on hemodynamics and cardiac function. Top: echocardiographic data (TM mode) are illustrating the changes in left ventricle (LV) diameters during infusion, along with the rapid drop in ejection fraction. Bottom: blood pressure, EKG signal, cardiac output and left ventricular pressure are shown. Within few minutes only, H_2S led to a complete pulseless electrical activity or electromechanical dissociation, as the heart becomes incapable of contracting despite a persistent electrical activity visible of the EKG signal. Modified from Refs. 19 and 20.

Figure 4.

Example of the effects of exposure to a lethal level of H_2S in one sheep. Recording is shown starting five minutes after the end of exposure. APB: arterial blood pressure; BF blood flow. Note that a picture of complete pulseless electrical activity or electromechanical dissociation occurred well after the end of exposure, i.e. at a time when free H_2S was undetectable in the blood or the expiratory gas.

Figure 5.

A. Recording obtained in one sheep during a continuous infusion of H_2S solution at a rate of $405 \mu\text{mol/min}$. APB: arterial blood pressure; FEH_2S : expired fraction of H_2S . The dashed vertical lines indicate the start and end of hydroxocobalamin (HyCo) injection (5 g in saline solution). The gaseous form of H_2S in the blood decreased dramatically during HyCo infusion reaching almost zero despite the persistence of sulfide infusion. B. Recording in one sheep during a continuous infusion of NaHS at the same rate of $405 \mu\text{mol/min}$, receiving an infusion of Methemoglobine (MetHb) solution (600 mg of human-derived Hb (Sigma Aldrich) in sterile saline (200 mg/mL)). Expired H_2S decreased dramatically during MetHb infusion and continued to decrease beyond the period of MetHb infusion. C. relationship between the mean $\pm$ SD concentrations of gaseous H_2S (CgH_2S) and total H_2S (CMBBH_2S), before and after MetHb infusion (black symbols) and HyCo infusion (open symbols). CgH_2S significantly decreased following MetHb infusion with a four-fold increase in CMBBH_2S reflecting the increased level of H_2S combined on MetHb. The decrease in CgH_2S was even more significant following HyCo infusion with no increase in CMBBH_2S , suggesting that H_2S oxidation may have been catalysed in the presence of the cobalt compound. Clearly although these two metallo-compounds do not have the same effect on the pools of combined H_2S , they both can decrease soluble H_2S very efficiently. Modified from Refs. 16 and 52.

Figure 6.

Recordings showing the effects of a continuous infusion of NaHS (10 micromoles/min) on arterial blood pressure, ventricular pressure and cardiac output (CO) in two rats. (A) Saline was injected intravenously as soon as cardiac output (CO) decreased by ~60%. CO, blood pressure and ventricular became undetectable at 530 seconds. (B) Injection of 2 mg MB IV, in another rat, induced a large increase in CO and blood pressure. A second MB injection was able to produce a similar effect late into the period of intoxication, delaying the moment of death, despite continuous sulfide infusion. (C) Mean $\pm$ SD values of left ventricular systolic pressure (LVSP) and dP/dt_{max} in rats receiving saline (open-circle) or 4 mg/kg MB (closed-circle) during lethal infusion of H_2S ($n = 14$, 10 μ mol/min). Saline and MB were injected at time 0 (dashed line); data were averaged every 10 seconds. Continuous H_2S infusion decreased cardiac function and LVSP. Saline injection did not affect the decrease in LVSP and dP/dt_{max} . In contrast, MB had an immediate effect on circulation, significantly increasing the cardiac function and circulation for about 2 minutes. #, significantly different from pre-injection values, $P < 0.05$. Modified from Ref. 19.