Arch Toxicol (1989) 63: 131–136

Archives of
Toxicology

Monoamine oxidase inhibition as a sequel of hydrogen sulfide intoxication: increases in brain catecholamine and 5-hydroxytryptamine levels

M. W. Warenycia, K. A. Smith, C. S. Blashko, S. B. Kombian, and R. J. Reiffenstein

Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7, Canada

Abstract. Administration of sodium hydrosulfide (NaHS), an alkali salt of hydrogen sulfide (H_2S) at doses of 10 and 30 mg/kg, corresponding to sublethal and lethal doses (0.66 and 2.0 X LD_{50}) resulted in significant increases in regional catecholamine levels of the rat brain only after the dose of 2.0 × LD_{50} of NaHS. Whereas the cortex and the cerebellum showed little or no change in catecholamine content, the hippocampus, striatum and brainstem all showed increases in noradrenaline and adrenaline. Additional analysis also showed that brainstem dopamine and 5-hydroxytryptamine levels (5-HT) increased as well. In vitro testing of sulfide for inhibition of monoamine oxidase (MAO) activity showed the anion to be inhibitory with an IC_{50} of 39.1 ± 3.6 μM. Inhibition of MAO activity ex vivo could be demonstrated at a dose of 100 mg/kg but not at the lower dose of 30 mg/kg NaHS. Inhibition of enzyme activity could not be demonstrated at this lower dose, possibly due to the well known rapid intramitochondrial metabolism of sulfide. Correlation of synaptosomal and mitochondrial sulfide levels with enzyme inhibition data suggests that inhibition of MAO may be an important contributing factor to the mechanism(s) underlying loss of central respiratory drive after fatal intoxication with H_2S.

Key words: Hydrogen sulfide – Catecholamines – 5-Hydroxytryptamine – Monoamine oxidase – Brainstem

Introduction

Although it has long been known that the predominant feature of hydrogen sulfide (H_2S) neurotoxicity involves loss of central respiratory drive (Evans 1967; Amman 1986), the basic mechanisms underlying the disruption of neurotransmission in brainstem nuclei controlling respiration remain to be elucidated. Sulfide toxicity has traditionally been ascribed to inhibition of mitochondrial oxidative phosphorylation by blockade of electron transport at the level of cytochrome aa_3 (Beauchamp et al. 1984).

In fact, H_2S has a greater demonstrated potency than cyanide in producing cytochrome inhibition at this locus (Nicholls 1975), although, paradoxically, sulfide and cyanide possess nearly identical LD_{50}'s (Persson et al. 1985; Warenycia et al. 1988). A further comparison of the two respiratory poisons also shows that regional changes in putative amino acid neurotransmitter levels are distinctly different as well (Persson et al. 1985; Kombian et al. 1988). In addition, the effects of cyanide on regional catecholamine levels appeared to be relatively minor and restricted to only one or two brain regions (Persson et al. 1985).

Since most of the catecholaminergic innervation of the brain originates from within brainstem structures (Dahlström and Fuxe 1971) and catecholamines as well as 5-hydroxytryptamine are known to markedly affect respiratory rhythm (Mueller et al. 1982; Murakoshi et al. 1985), the present investigation focused on the effects of sulfide on regional levels of the biogenic amines. In addition, the effects of sulfide on the activity of the important catabolic enzyme of monoamines, monoamine oxidase (MAO, EC 1.4.3.4) were also examinde in vitro as well as ex vivo.

Materials and methods

Sulfide source. Since H_2S can be generated in vivo by the administration of any of the alkali salts of sulfide (Beauchamp et al. 1984), sodium hydrosulfide, NaHS (Aldrich Chemical Co., Milwaukee, WI) was used exclusively throughout this study. Prior to use in animal studies, iodometric titration (Standard Methods 1985) of the NaHS stock showed that the salt was in the form of the dihydrate. Accordingly, all dosages of NaHS express the concentration of hydrosulfide in terms of the unhydrated salt. The LD_{50} for sodium hydrosulfide in the adult male Sprague-Dawley rats (250–350 g) used in the experiments cited herein was previously determined (Warenycia et al. 1988) by probit analysis (Cook 1972) to be approximately 15 mg/kg i.p. (LD_{50} = 14.7 ± 0.9 mg/kg; mean ± S.D.; n = 100).

Brain regional studies. Animals received either saline, 10 mg/kg NaHS (0.66 × LD_{50}) or 30 mg/kg NaHS (2 × LD_{50}) by intraperitoneal injection. Animals not succumbing to lethal injections of NaHS were subjected to cervical dislocation 2 min after administration of NaHS or saline. The 2 min time was chosen, since this was the median time to death in LD_{50} studies. Brains were removed, rinsed in ice-cold saline and dissected on ice into brainstem, cerebellum, hippocampus, striatum and cortex as previously described (Kombian et al. 1988; Warenycia et al. 1988). Brain regions were then individually weighed and then sonicated in 4–10 volumes of ice-cold 0.4 N $HClO_4$. The

Offprint requests to: M. W. Warenycia

dissection, weighing, and sonication procedure took approximately 5 min. Sonicates were then centrifuged for 5 min at 11800 *g* and the supernatants transferred to fresh sample vials. All samples were then deep frozen (−80° C) until analysis 2–7 days later.

Catecholamine and 5-hydroxytryptamine analyses. Aliquots, or suitable dilutions thereof, from the various brain region supernatants were analyzed for catecholamines by High Performance Liquid Chromatography (HPLC) with electrochemical detection. The column employed was a reverse-phase C_{18} column and the mobile phase consisted of 0.1 M Na_2HPO_4, 0.1 M sodium citrate, 0.1 mM EDTA and 100 mg/l sodium octyl sulphate, pH 4.0. Authentic sets of standards containing dopamine, noradrenaline, and adrenaline were run in each analysis. The elution order was: noradrenaline < adrenaline < dopamine. Interference by sulfide with electrochemical detection for catecholamines was considered unlikely, since the applied potential at the working electrode with respect to an Ag/AgCl reference electrode was +0.72 V. At this applied potential the contribution of any sulfide ion present would be negligible as previous electrochemical studies have shown sulfide oxidation to be maximal at the much lower applied potentials of 0.00 to 0.06 V (Goodwin et al. 1988).

Supernatants of brainstem samples corresponding to control (i.e. saline-treated) and 30 mg/kg NaHS were also analyzed by HPLC for 5-hydroxytryptamine (5-HT). For these determinations the mobile phase consisted of: 15 mM NaH_2PO_4, 0.73 mM sodium octyl sulphate, 0.37 mM EDTA and 9% (v/v) acetonitrile, pH 3.0. Quantitation was achieved by the use of the internal standard dihydroxybenzylamine (20 ng) added to each supernatant. Authentic standards of 5-HT were also run to define the elution time, which established that 5-HT was the last peak eluted.

Sulfide analysis. Synaptosome and mitochondrial-enriched fractions were prepared according to the method of Hajos (1975). Brains from control or 50 mg/kg NaHS-treated animals were homogenized in 10 volumes of 0.32 M sucrose dissolved in 0.01 NaOH to reduce H_2S volatilization. Homogenates were then centrifuged at 1000 *g* for 10 min. The supernatants were decanted and then centrifuged at 10000 *g* for 20 min. The pellet was resuspended in fresh 0.32 M sucrose and further purified by density gradient centrifugation at 8000 *g* for 30 min. Synaptosomal and mitochondrial-enriched fractions, corresponding to the 0.8 M sucrose layer and the pellet, were collected. The volume of the former was 20.0 ml and the pellet was resuspended in 10 ml 0.01 M NaOH. Zinc acetate (135 or 67.5 µl of a 1.0 M stock solution, respectively) was then added to each subcellular fraction to trap sulfide as the insoluble zinc sulfide precipitate.

Fractions were then analyzed for sulfide by gas dialysis and ion chromatography as described recently (Goodwin et al. 1988; Warenycia et al. 1988). Briefly, each samples was treated with 6 M HCl to liberate sulfide from the zinc precipitate in a Technicon gas dialysis membrane unit; liberated H_2S was then absorbed into fresh 0.01 M NaOH. An aliquot of this solution was then applied onto a Dionex fast anion separator column (type AS3) and sulfide detected and quantitated electrochemically using authentic standards of sodium sulfide.

Monoamine oxidase assay (EC 1.4.3.4). Monoamine oxidase activity of whole rat brain was estimated by the radiometric assay of Wu and Dyck (1977) using [C^{14}]-tyramine (New England Nuclear, 40.5 mCi/mmol) as the substrate. Whole brains from saline-injected rats (controls) or those treated with NaHS (30 or 100 mg/kg i.p.) were weighed and individually homogenized in 10 volumes of ice-cold phosphate (50 mM) buffer, pH 7.4, containing 0.2% (v/v) Triton X-100 (J. T. Baker Chem Co.) using a Brinkmann Polytron at a setting of 5 for approximately 3–4 s. Homogenates were then further diluted five-fold with phosphate buffer prior to use in the enzyme assay.

The final assay volume (per 1.5 ml Eppendorf tube) consisted of: 50 µl of the diluted brain homogenate as the source of the enzyme, 130 or 140 µl of phosphate buffer and 10 µl of [C^{14}]-tyramine at a final concentration of 2.2 µM. In each experiment a set of tubes containing pargyline, a known MAO inhibitor, were included. The final concentration of pargyline (added as a 10 µl aliquot) was 5×10^{-6} M (5.0 µM). In experiments designed to determine whether sulfide or hydrosulfide anion could affect enzyme activity in vitro, 10 µl aliquots from appropriate stock solutions of NaHS were added to assay tubes to achieve final concentrations in the 5–160 µM range.

The enzyme reaction was carried out at 37° C for 1 h with gentle shaking in a water bath and the reaction slowed by immersing tubes in an ice water bath. Under the conditions described and at a homogenate dilution of 1:50 (v/v), the formation of the oxidized product by the enzyme was linear with respect to time and substrate concentration. Under the exact conditions of this assay sulfide volatilization (measured using the technique of Goodwin et al. 1988) was approximately 90% of the initial concentration, i.e. only 10% remained at the end of 1 h.

The enzyme reaction was terminated by the addition of 500 µl chloroform containing the liquid-ion exchanger, diethyl hexylphosphoric acid (0.1 M; Sigma Chemical Co., St Louis, MO.) to each tube. Tubes were vortexed for 15 s and then centrifuged at 4000 *g* for 5 min. A 100 µl aliquot of each aqueous phase was then carefully aspirated and transferred to scintillation vials containing 0.5 ml 0.1 N $HClO_4$. To each vial was added 10 ml of a toluene-based fluor (PPO-POPOP; ICN Radiochemicals). Samples were tightly capped and then vortexed for 30 s. Radioactivity was quantitated using an external standard with automatic correction for chemiluminescence (LKB instruments). Counting efficiency was routinely greater than 85%.

Protein determination. In this study it was not possible to accurately quantitate the protein content of whole brain homogenates from NaHS-treated animals using Hartree's method (1972), because of unexpected interference by sulfide, resulting in increased color development. However, since all brains were homogenized in 10 volumes of buffer (w/v) and determination of protein was straightforward in control brain homogenates, results for NaHS-derived brain homogenates are expressed as % of control as this represents a reasonable first-order approximation to base comparisons of enzyme activity. Secondly, only control brain homogenates were used for determining possible inhibitory effects of hydrosulfide or sulfide anion in vitro and an aliquot of each homogenate was saved for subsequent protein determination prior to the addition of any sulfide.

Statistical analysis. Catecholamine levels in the various brain regions were compared by analysis of variance and Duncan's New Multiple Range Test (Dowdy and Weardon 1983). A p value of less than 0.05 was considered significant. Changes in enzyme activity between controls and NaHS-treated animals were compared using the unpaired Student's t-test. The IC_{50} value for sulfide in vitro inhibition tests was calculated by probit analysis (Cook 1972). Sulfide concentrations in synaptosomal and mitochondrial fractions were also compared using the unpaired t-test.

Results

No changes in catecholamine levels were seen in the cortex at either 10 mg/kg or 30 mg/kg NaHS (Table 1). In the cerebellum only dopamine levels increased, to 140% of control in response to 30 mg/kg NaHS, as shown in Table 1. In the other brain regions (the hippocampus, the striatum, and the brainstem) noradrenaline and adrenaline both increased at 30 mg/kg NaHS as compared to saline-injected controls (Table 2). The magnitude of the increases was greatest in the hippocampus: 268% and 356% for noradrenaline and adrenaline as compared to their respective control levels. Furthermore, unlike in the other brain regions, a statistically significant elevation of hippocampal noradrenaline (165% of controls) could also be demonstrated at the lower dose of 10 mg/kg NaHS.

Brainstem increases of noradrenaline and adrenaline at 30 mg/kg NaHS were 202% and 233%, respectively, when compared to the saline-injected controls. In addition, levels of dopamine also increased in the brainstem by 326% as compared to the controls. The smallest increases in noradrenaline and adrenaline levels (131% and 171%, respectively) occurred in the striatum following 30 mg/kg NaHS. The results for hippocampus, brainstem and striatum are summarized in Table 2. Further analysis of brainstem samples by an alternative HPLC protocol also showed considerable increases in the levels of 5-hydroxytryptamine (383% as compared to controls).

In vitro inhibition of monoamine oxidase activity by sulfide was readily demonstrable at concentrations above 20 μM. Inhibition was linearly related to the logarithm of added sulfide with an IC_{50} of 39.1 ± 3.6 μM as determined by probit analysis (Fig. 1). Determination of the sulfide content of synaptosomal and mitochondrial-enriched fractions for controls and the dose of 50 mg/kg NaHS is given in Table 3. Extrapolation of the in vivo sulfide concentration as measured in mitochondria to the in vitro inhibition data of Fig. 1 results in an a priori prediction of 15–20% inhibition of MAO activity at the dose of 30 mg/kg NaHS, and 40–50% inhibition of enzyme activity at the higher dose of NaHS. However, inhibition of MAO activity after i.p. administration of 30 mg/kg NaHS could not be demonstrated. Furthermore, the interval between respiratory arrest and homogenization and subsequent assay did not appear to be a critical factor at this dose as similar results were obtained with time intervals of 3 and 15 min (Fig. 2). At the much higher NaHS dose of 100 mg/kg approximately a 47% inhibition of MAO activity could be demonstrated (Fig. 2). Throughout these experiments pargyline at a concentration of 5×10^{-6} M, nonetheless still produced 50–70% inhibition of MAO activity as shown in Fig. 2. By comparison to intramitochondrial sulfide content at an NaHS dose of 50 mg/kg and the in vitro data

Table 1. Lack of effect of NaHS treatment on catecholamine levels in the cortex and the cerebellum[a]

	Sulfide dose		
	Controls[b]	10 mg/kg	30 mg/kg
Cortex			
Dopamine	0.21 ± 0.01	0.20 ± 0.04	0.16 ± 0.03
Noradrenaline	0.28 ± 0.09	0.24 ± 0.06	0.38 ± 0.14
Adrenaline	0.17 ± 0.04	0.22 ± 0.03	0.22 ± 0.04
Cerebellum			
Dopamine	0.35 ± 0.04	0.39 ± 0.02	0.49 ± 0.03[c]
Noradrenaline	2.47 ± 0.24	2.09 ± 0.26	2.07 ± 0.32
Adrenaline	1.20 ± 0.13	1.15 ± 0.07	1.40 ± 0.17

[a] All values expressed as the mean ± SE in μg/g wet weight. $N = 5$ in each group
[b] Received saline only
[c] $p < 0.05$ compared to controls (Duncan's Multiple Range Test)

Table 2. Increases in catecholamine levels in hippocampus, brainstem, and striatum following NaHS treatment[a]

	Sulfide dose		
	Controls[b]	10 mg/kg	30 mg/kg
Hippocampus			
Dopamine	0.81 ± 0.19	1.11 ± 0.09	0.98 ± 0.20
Noradrenaline	1.09 ± 0.10	1.45 ± 0.19[d]	2.94 ± 0.73[d]
Adrenaline	0.51 ± 0.07	0.78 ± 0.08	1.82 ± 0.30[d]
Brainstem[c]			
Dopamine	0.57 ± 0.06	0.60 ± 0.04	1.85 ± 0.25[d]
Noradrenaline	0.90 ± 0.13	1.25 ± 0.12	1.82 ± 0.12[d]
Adrenaline	0.43 ± 0.03	0.62 ± 0.05	1.26 ± 0.08[d]
Striatum			
Dopamine	6.59 ± 0.48	5.40 ± 0.09	6.37 ± 0.34
Noradrenaline	1.78 ± 0.06	1.96 ± 0.20	2.33 ± 0.33[d]
Adrenaline	1.13 ± 0.18	1.32 ± 0.16	1.89 ± 0.52[d]

[a] All values given in μg/g wet weight expressed as the mean ± SE; $N = 5$ in each group
[b] Received only saline i.p.
[c] 5-HT levels in control brainstem (0.29 ± 0.08 μg/g) also differed significantly ($p < 0.001$, Student's t-test) from 5-HT levels at 30 mg/kg NaHS (1.11 ± 0.13 μg/g)
[d] $p < 0.05$, compared to controls using Duncan's Multiple Range Test

Table 3. Sulfide concentration within synaptosomal and mitochondrial-enriched fractions of rat brain before and after 50 mg/kg NaHS[a]

	Sulfide (μM)	
	Controls[b]	50 mg/kg NaHS
Synaptosomal fraction	1.00 ± 0.003	2.13 ± 0.02[c]
Mitochondrial fraction	5.25 ± 0.003	12.09 ± 0.10[d]

[a] Sulfide concentration expressed as μM with $N = 4$ for each group; results given as mean ± SE
[b] Received saline only
[c] $p < 0.05$
[d] $p < 0.01$; Student's t-test

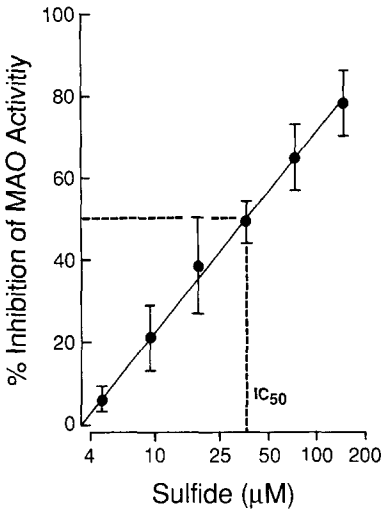

Fig. 1. Inhibition of rat whole brain monoamine oxidase activity by sulfide in vitro. Each *point* represents the mean ± SE of 4–8 determinations. Enzyme activity for controls (no added sulfide) was 0.56±0.05 nmole/mg protein/min (mean ± SE; $N = 8$) of deaminated product formed. The IC_{50} was 39.1±3.6 μM as determined by probit analysis

showing MAO inhibition by sulfide, it is apparent that there is very close agreement between measured and predicted values for MAO inhibition.

Discussion

The brainstem catecholamine levels of control animals in this study agree closely with other recent work (Foster et al. 1987) and may indicate that sulfide-induced increases in catecholamines occur in a neurochemically separate neuronal population. Earlier work had already suggested ascending adrenergic inhibitory control over brainstem noradrenergic cells (Astier et al. 1986). Disruption of this circuit by sulfide may be detrimental to the pontine and medullary neurons that pattern central respiratory drive (Segers et al. 1985). The sulfide sensitivity of these catecholaminergic neurons may be related to selective accumulation of sulfide by the brainstem (Warenycia et al. 1988) due to increased H_2S solubility (Windholz 1976) in lipid-enriched brain regions.

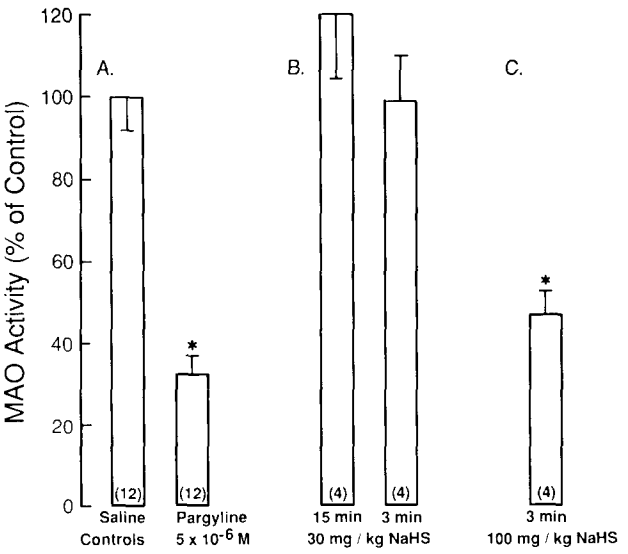

Fig. 2. Whole rat brain monoamine oxidase activity in NaHS-treated animals. *A* Control (saline-injected) and pargyline (5×10^{-6} M) added in vitro; *N* indicated by numbers in parentheses. *B* 30 mg/kg NaHS ($2 \times LD_{50}$) with brains homogenized 3 or 15 min after death. *C* 100 mg/kg NaHS ($6.66 \times LD_{50}$) with brains homogenized 3 min after death. All values expressed as % of control and given as mean ± SE. Control value for MAO activity was 0.63±0.05 nmole/mg protein/min (mean ± SE; $N = 12$). * $p < 0.001$ by Student's *t*-test

This study demonstrates that a dose of twice the LD_{50} of NaHS markedly elevates noradrenaline and adrenaline levels in the hippocampus, striatum and brainstem but not in the cerebellum or cerebral cortex. The greatest increases following NaHS occurred in the hippocampus, and may be associated with the amnesia reported following H_2S intoxication (Burnett et al. 1977). In the dopamine-rich striatum only noradrenaline and adrenaline were elevated. Although the effect of these increases on striatal dopaminergic neurotransmission is unknown, impairment of extrapyramidal function could contribute to the apparent inability of H_2S-knockdown victims to escape heavily-laden H_2S environments (Burnett et al. 1977).

Of greatest consequence, however, are increased brainstem catecholamine and 5-HT levels following a lethal NaHS dose since these may be directly contributory to loss of central respiratory drive after H_2S. The major catecholaminergic nuclei are located in the brainstem, (Dahlstrom and Fuxe 1964), and catecholamines as well as 5-HT influence the respiratory rhythm (Mueller et al. 1982; Murakoshi et al. 1985). Increases in dopamine may have occurred in brainstem adrenergic neurons. Furthermore, in brain regions lacking an adrenergic but having a noradrenergic innervation (i.e., hippocampus and striatum) dopamine levels remained unchanged, suggesting that the increases in brainstem dopamine levels are restricted only to adrenergic neurons. Isoenzyme forms of tyrosine hydroxylase have been previously postulated (Raese et al. 1977), thus making it possible that sulfide very rapidly affects this enzyme only within adrenergic neurons.

Tyrosine hydroxylase activation by sulfide may be related to increases in catecholamine biosynthesis seen after hypercarbia where intraneuronal pH changes or chemoreceptor stimulation modify central catecholaminergic activity (Davis and Carlsson 1973; Garcia DeYebenes Prous et al. 1977). In this regard, hydrosulfide anion may cause acidosis by inhibiting carbonic anhydrase (Klentz and Fedde 1978), and H_2S stimulates peripheral chemoreceptors (Amman 1986). Higher noradrenaline or adrenaline levels could also be due to increased activity of either dopamine-β-hydroxylase (DBH; EC 1.14.2.1) or phenylethanolamine-N-methyl transferase (PNMT; EC 2.1.1.28). Increased DBH activity is unlikely, since an endogenous DBH inhibitor that is activated by H_2S has already previously been reported (Duch and Kirshner 1971). Whether PNMT activity can be stimulated by sulfide has yet to be determined. Lastly, there is also some evidence for ancillary pathways of adrenaline biosynthesis independent of tyrosine hydroxylase (Foster et al. 1987).

The rapid increases in catecholamines may be best explained by inhibition of the degradative enzymes of monamine metabolism, either catechol-O-methyl transferase (COMT; EC 2.1.1.6) or monoamine oxidase (MAO; EC

1.4.3.4). Although COMT activity was not examined in the present study, the sensitivity of COMT to sulfhydryl reagents (Borchardt 1977), suggests it is susceptible to sulfide inhibition. MAO inhibition by sulfide, both in vitro and at a dose of 100 mg/kg of NaHS, with an IC_{50} within the range of mitochondrial sulfide levels, was not unexpected, since MAO is a mitochondrial enzyme (Nagatsu et al. 1977). Furthermore, the presence of sulfide in mitochondrial protein fractions (Miller 1970) makes it possible that MAO activity in vivo may be partially regulated by endogenous sulfide.

The active site of MAO contains multiple sulfhydryl groups (Yasunobu and Oi 1972), which may be vulnerable to persulfide formation. The inability to detect MAO inhibition at the lower 30 mg/kg dose of NaHS, a dose that nonetheless produced lethality as well as elevations of brain catecholamines, may reflect the well-known extremely rapid intramitochondrial metabolism of sulfide (Banki et al. 1986). At a 100 mg/kg dose of NaHS saturation of sulfide-metabolizing capability has been achieved, leaving enough sulfide to demonstrably inhibit MAO ex vivo. Furthermore, the results obtained using ex vivo measurement of MAO activity are consistent with other studies (Waldmeier et al. 1984) in that higher doses of NaHS than used in vivo were required to demonstrate inhibition. MAO inhibition in ex vivo experiments may be underestimated due to dilution of tissue homogenates and subsequent dissociation of the enzyme-inhibitor complex, a problem further compounded by the ready volatility of H_2S.

MAO inhibition, and monoamine increases in the brainstem may be important in the loss of central respiratory drive after H_2S exposure. The extent of inhibition seen in vivo can be accurately correlated to the percent inhibition in vitro if the dose is high enough to compensate for rapid sulfide detoxification. Although the effects of MAO inhibition on central respiratory drive are unknown, MAO inhibitors such as clorgyline or deprenyl (quoted by Mueller et al. 1982) change the dose-dependent biphasic effects of dopamine. It is thus noteworthy that increases in respiratory frequency characterize the early stages of H_2S intoxication (Amman 1986).

The presence of an endogenous brainstem sulfide level (Warenycia et al. 1988) may therefore be of importance in neonatal apnea syndromes as suggested by others (Mueller et al. 1982). Abnormalities in sulfide metabolism could result in altered monoaminergic neurotransmission. Additional work, concentrating on the increased responsiveness of catecholaminergic neurons to hydrogen sulfide, and the effects on central respiratory drive is therefore required.

Acknowledgements. This study was supported by Alberta Community and Occupational health through the Heritage Trust Grant Program. The technical assistance of Frank Mele, Karen Fung and Donna Francom is appreciated. The authors wish to thank Dr Glen Baker for generously providing pargyline and the 5-HT analyses. The helpful suggestions of Dr G. M. McKenzie and Dr W. Dryden are gratefully acknowledged, as well as the invaluable comments of Dr. J. D. Taylor on the manuscript. The skillful drafting of Tina Cho and the wordprocessing skills of Margaret Carmichael are greatly valued. We wish to especially thank Lorne Goodwin at the Alberta Environmental Centre (Vegreville) for generous assistance with the sulfide analyses, and Dr C. Benishin (Physiology) for aid in synaptosome preparation.

References

Amman HJ (1986) A new look at physiologic respiratory response to hydrogen sulfide poisoning. J Hazard Mat 13: 369–374

Astier B, Kitahama K, Denoroy L, Berod A, Jouvet M, Renaud B (1986) Biochemical evidence for an interaction between adrenaline and noradrenaline neurons in the rat brainstem. Brain Res 397: 333–340

Banki K, Elfarra AA, Lash LH, Anders MW (1986) Metabolism of *S*-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine in *S*-(2-chloro-1,1,2-trifluroethyl)-L-cysteine-induced mitochondrial toxicity. Biochem Biophys Res Commun 138: 707–713

Beauchamp RO, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA (1984) A critical review of the literature on hydrogen sulfide toxicity. CRC Crit Rev Toxicol 13: 25–97

Borchardt RT, Thakker DR (1976) Evidence for sulfhydryl groups at the active site of catechol-O-methyltransferase. Biochem Biophys Acta 445: 598–609

Burnett WW, King EG, Grace M, Hall WF (1977) Hydrogen sulfide poisoning; review of 5 years experience. J Can med Assoc 117: 1277–1280

Cook DA (1972) The use of the APL 360 system in pharmacology. Part II. Probit analysis. Comput Biomed Res 5: 347–350

Dahlström A, Fuxe K (1964) Evidence for the existence of monoamines-containing neurons in the central nervous system. I.-Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiol Scand 62: Suppl 232:1–15

Davis J, Carlsson A (1973) The effect of hypoxia on monoamine synthesis levels and metabolism in rat brain. J Neurochem 21: 783–790

Dowdy S, Weardon S (1984) Statistics for research. John Wiley & Sons, New York

Duch DS, Kirshner N (1971) Isolation and partial characterization of an endogenous inhibitor of dopamine β-hydroxylase. Biochem Biophys Acta 236: 628–638

Evans CL (1967) The toxicity of hydrogen sulfide and other sulfides. J Exp Physiol 52: 231–248

Foster GA, Sundström E, Helmer-Matyjek E, Goldstein M, Hökfelt T (1987) Abundance in the embryonic brainstem of adrenaline during the absence of detectable tyrosine hydroxylase activity. J Neurochem 48: 202–207

Garcia DeYebenes Prous J, Carlsson A, Gomez MA (1977) The effect of CO_2 on monoamine metabolism in rat brain. Naunyn-Schmiedebergs Arch Pharmakol 301: 11–15

Goodwin LR, Francom D, Urso A, Dieken FP (1988) Determination of trace sulfides in turbid waters by gas dialysis/ion chromatography. Anal Chem 60: 216–219

Hajos F (1975) Improved method for preparation of synamptosomal fractions n high purity. Brain Res 93: 485–489

Hartree EF (1972) Determination of protein: a modification of lowry method that gives a linear photometric response. Anal Biochem 48: 422–427

Klentz RD, Fedde MR (1978) Hydrogen sulfide: effects on avian respiratory control and intrapulmonary CO_2 receptors. Respir Physiol 32: 355–367

Kombian SB, Warenycia MW, Mele F, Reiffenstein RJ (1988) Effects of acute intoxication with hydrogen sulfide on central amino acid transmitter systems. Neurotoxicology 9: 587–596

Miller RW (1970) Estimation of labile sulfide content of cellular components. Anal Biochem 35: 181–192

Mueller RA, Lundberg DBA, Breese GR, Hedner J, Hedner T, Jonason J (1982) The neuropharmacology of respiratory control. Pharmacol Rev 34: 255–285

Murakoshi T, Suzue T, Tamai S (1985) A pharmacological study on respiratory rhythm in the isolated brainstem-spinal cord preparation of the newborn rat. Br J Pharmacol 86: 95–104

Nagatsu T, Nakano G, Mizutani K, Harada M (1972) Purification and properties of amine oxidases in brain and connective tissue (dental pulp). In: Costa E, Sandler M (eds) Monamine Oxidases – new vistas. Advances in Biochemical Psychopharmacology, Vol 5, Raven press, New York, p 29

Nicholls P (1975) The effect of sulfide on cytochrome aa_3, isosteric and allosteric shifts of the reduced α_1-peak. Biochem Biophys Acta 396: 24–35

Persson SA, Cassel G, Selstrom A (1985) Acute cyanide intoxication and central transmitter systems. Fund Appl Toxicol 5: S150–S159

Raese JD, Edelman AM, Lazar MA, Barchas JD (1977) Bovine striatal tyrosine hydroxylase: multiple forms and evidence for phosphorylation by cyclic AMP-dependent protein kinase. In: Usdin E, Weiner N, Youdim MBH (eds) Structure and function of monoamine enzymes. Modern Pharmacology-Toxicology Vol 10. Marcel Dekker Inc., New York, Basel pp 383–400

Segers LS, Shannon R, Lindsey BG (1985) Interactions between rostral pontine and ventral medullary respiratory neurons. J Neurophysiol 54: 318–334

Standard methods for examination of waste and waste water (1985) 16th Edition, APHA, AWWA WPCF, Washington

Waldmeier PC, Felner AE, Lauber J (1984) Reversible monoamine oxidase inhibitors: relation between affects on enzymatic activity measured ex vivo and on amine metabolism. In: Tipton KF, Dostert P, Strolin Benedetti M (eds) Monoamine oxidase and disease, Academic Press, New York, pp 107–116)

Warenycia MW, Goodwin LR, Francom D, Reiffenstein RJ, Dieken FP, Taylor J (1988) Regional brain distribution of sulfide after H_2S poisoning. In: Jones G, Singer P (eds). Twenty-fourth Annual Meeting of the International Association of Forensic Toxicologists, Banff, Canada. University of Alberta Press, Edmonton, pp 357–364

Windholz M (1976) Hydrogen sulfide. In: The Merck Index, 10th Edn. Merck and Co., Rahway, New Jersey, p 696

Wu PH, Dyck LE (1976) Microassay for the estimation of monoamine oxidase activity. Anal Biochem 72: 637–642

Yasunobu KT, Oi S (1972) Mechanistic aspects of the bovine hepatic monoamine oxidase reaction. In: Costa E, Sandler M (eds) Monoamine oxidases – new vistas. Advances in Biochemical Psychopharmacology Vol 5, Raven Press, New York, pp 91–104

Received June 27, 1988/Returned after revision October 12, 1988/Accepted October 31, 1988