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HYDROGEN SULFIDE SPECIFICALLY ALTERS NAD(P)H QUINONE DEHYDROGENASE 1 (NQO1) OLFATORY NEURONS IN THE RAT

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Abstract

The regions of the olfactory epithelium affected by hydrogen sulfide (H₂S) toxicity in the rat present a striking similarity with the developmental olfactory zone 1 described in the mouse. This zone which is the only region containing neurons expressing NAD(P)H quinone dehydrogenase 1 (NQO1) is involved in complex behavioral responses in rodents, and other mammals, triggered by specific olfactory stimuli. We therefore sought to determine whether 1) olfactory neurons expressing NQO1 are located in the same regions in the rats and in the mice and 2) there is an overlap between olfactory neurons expressing this protein and those affected by the toxicity of H₂S. Rats were exposed to H₂S – 200 ppm during 3 hours, 3 consecutive days- and displayed symmetric acute segmental necrosis of the neurons and sustentacular cells of the olfactory epithelium in the dorsomedial nasal cavity. We found that expression of NQO1 in Sprague-Dawley rats spatially recapitulated that of the mouse. The degree of agreement or overlap between these 2 populations of neurons (necrosis vs. NQO1 expression) reached 80.2 %. Although the underlying mechanisms accounted for the high sensitivity for NQO1 neurons -or the relative protection of non NQO1 neurons- to sulfide toxicity remain to be established, this observation is offering an intriguing approach that could be used to acutely suppress the pool of neural cells in olfactory zone I and to understand the mechanisms of toxicity and protection of other populations of neurons exposed to sulfide.

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INTRODUCTION

H₂S remains a primary chemical occupational exposure hazard in oil production, well drilling, gas refining industries (Arnold et al., 1985), as well as in farming (Chenard et al., 2003). Anosmia has long been reported, among other neurological deficits, following acute exposure to hydrogen sulfide (H₂S) toxicity (Tvedt et al., 1991).

Experimental inhalation of H₂S in the rat provokes discrete well-demarcated necrotizing lesions of the sensory neurons and sustentacular cells of the nasal olfactory epithelium in a dose- and time-dependent manner (Lopez et al., 1988; Brenneman et al., 2000; Brenneman et al., 2002; Dorman et al., 2004). For instance, no obvious lesion appears to be produced at levels of H₂S below 20 ppm even after long periods of exposure (Dorman et al., 2004). In contrast, exposing rats to H₂S for weeks at a concentration of 30 or 80 ppm induces significant lesions of the olfactory epithelium (Brenneman et al., 2000; Dorman et al., 2004), while exposure to H₂S between 200–400 ppm leads to an extensive olfactory epithelium necrosis after only a few hours (Lopez et al., 1988; Brenneman et al., 2002). Some of the remarkable features of these lesions have already been noticed (Lopez et al., 1988): 1) H₂S primarily affects the sensory neurons and sustentacular cells (Brenneman et al., 2002) and not the basal cells, the latter of which becomes necrotic only at concentrations that would provoke systemic effects (400 ppm and above) and 2) only certain regions of the olfactory epithelium are affected, with a sharp demarcation between full necrosis and intact epithelium. Indeed, it is predominantly the olfactory epithelium present in the dorsal meatus that appears to be affected (Brenneman et al., 2000; Brenneman et al., 2002).

The extreme sensitivity of the olfactory neurons to H₂S intoxication is not unexpected by analogy to the behavior of other neurons in response to a reduction in ATP production, whether in the presence of cyanide, anoxia or H₂S. In addition, neurons, at least in the central nervous system, seem to lack expression of the enzyme sulfide quinone reductase (SQR) (Lagoutte et al., 2010; Linden et al., 2012), one of the main mechanisms of H₂S oxidization/detoxification in the mitochondria.

However, the specific anatomical distribution of the H₂S-induced lesions of the olfactory neurons in the dorsal region of the olfactory epithelium remains difficult to explain. Regional differences in H₂S flows and interactions with the nasal epithelium have been proposed to account for such a distribution of the lesions, based on original computational model studies of nasal air flow (Moulin et al., 2002; Schroeter et al., 2006a). However, this analysis could not fully explain the discrete and specific “dorsal” predilection of sulfide toxicity, including the sharp demarcation with intact adjacent olfactory epithelium.

We were intrigued by some of our own data as well as the data discussed by Moulin et al. and Schroeter et al. (Moulin et al., 2002; Schroeter et al., 2006a), who combined experimental data from Brenneman et al (Brenneman et al., 2002) with a computational model of H₂S distribution in the nose, showing that the regions of olfactory epithelial necrosis presents a striking similarity with the olfactory zone 1 described in the mouse (Ressler et al., 1993). This zone comprises a pool of olfactory neurons that have a different embryological origin and gene expression pattern, which follows a clear dorso-ventral and

rostral caudal somatotopy (Ressler et al., 1993; Scott and Brierley, 1999). Zone 1 contains neurons which make synaptic connection with the most dorsal regions of the olfactory bulbs (Mori et al., 1999) and can trigger various systemic responses, including the fear response in mice (Dewan et al., 2013). Zone 1 neurons not only express specific olfactory receptors (Zhang et al., 2004) but also non-receptor proteins absent from other olfactory neurons, particularly NAD(P)H quinone dehydrogenase 1 (NQO1) (Gussing and Bohm, 2004).

In keeping with this intriguing similarity, we sought to establish in the rat whether the necrotic lesions produced by H₂S preferentially involve neurons located in zone 1, as determined by co-localization with NQO1 expression. Addressing this question has very practical implications, as H₂S inhalation induced neuronal necrosis could be used to produce and study animal models deprived from olfactory neurons in zone 1, while the rest of the olfactory epithelium would be preserved. In addition, since H₂S-induced neuronal necrosis produces a striking demarcation between healthy and necrotic neurons in the central nervous system (Sonobe et al., 2015), the present study may have relevance outside the olfactory epithelium.

MATERIALS AND METHODS

Fourteen male Sprague-Dawley rats (Crl:SD, strain code 400, Charles River Laboratories) weighing 403 ± 92 g (~12–16 weeks) were studied. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition (National Research Council (US) Institute for Laboratory Animal Research). The study was approved by the Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee. All rats were housed in open top cages in a temperature-controlled conventional room ($24 \pm 1^\circ\text{C}$) with 12-hour light/dark cycle, and were provided with rodent chow (Envigo 2108, Teklad global, 18% protein) and water ad libitum.

In keeping with previously published studies, we choose to expose the animals to 200 ppm H₂S for 3 hours during 3 consecutive days (Brenneman et al., 2002). Rats were totally randomly selected, while the pathologist was blinded to the procedure and the nature of the exposure.

The animals were placed in a large chamber (25–25–40 cm) and were acclimatized for at least a week prior to the experiments, using a similar protocol of exposure while the chamber was only flushed with air. Exposure to 200 ppm H₂S was performed using a calibrated compressed H₂S mixture (Certified standard UN 1956, H₂S, 200 ppm balance air, PRXAIR distribution, Geismar LA USA) which flow was adjusted to 6 l/min using a precision rotameter. Gas was delivered through the inlet port of the chamber. H₂S concentration was continuously measured from both the inlet and outlet ports, by using H₂S analyzers (Interscan RM series, Interscan Corporation, Simi Valley, CA. In addition, the levels of O₂ and CO₂ were continuously monitored in the outlet port (Oxystar-100, CWE Inc. Ardmore, PA, model 17630, VacuMed, Ventura, CA). The flow of gas (V) entering the chamber was determined by pneumotachograph (1100 Series, Hans Rudolph, Inc. Shawnee, KS). Eight animals were exposed at the same time to H₂S for 3 hours at a time for 3 consecutive days, while an additional 3 animals were only exposed to air.

Rats were sacrificed at two (n=3) or three (n=5) days after the last H₂S exposure. Air-exposed rats (n=3) were sacrificed at two days post-exposure. Rats were anesthetized with 3.5% isoflurane in O₂ followed by urethane (1.2 g/kg, IP). Thirty minutes later, the animals were perfused transcardially with phosphate buffered saline (PBS) followed by 10% neutral buffered formalin (NBF). Heads were post-fixed and decalcified in formic acid (Formical-4, StatLab Medical Products, McKinney, TX), and seven transverse sections through the nasal cavity were made for each rat. Tissues were processed in an automated Tissue-Tek VIP processor and paraffin-embedded with a Tissue-Tek TEC embedding station (Sakura Finetek USA, Torrance, CA). Sections were cut at 6 µm for routine hematoxylin and eosin (H&E) staining or were mounted on charged plus slides for immunohistochemistry (IHC). All tissues were examined by an ACVP diplomate pathologist blinded to treatment. All H&E stained images were obtained with an Olympus BX51 microscope and DP71 digital camera using cellSens Standard 1.12 imaging software (Olympus America, Center Valley, PA).

The anatomical distribution of neurons expressing NQO1 and OMP (olfactory marker protein) was evaluated with IHC. Primary antibodies used for IHC were anti-NQO1 (Abcam; goat, 1:1000; Cambridge, MA) and anti-OMP (Wako; goat, 1:1000; Richmond, VA). Donkey anti-goat IgG conjugated with Alexa 488 (Thermo Fisher Scientific, Waltham, MA) was used as secondary antibody. Following deparaffinization, the slices were pretreated with TBS-T (10 mM Tris-HCl (pH 7.4), 100 mM NaCl with 0.3% Triton-X100 (vol/vol)), and blocked with blocking buffer (5% normal donkey serum (vol/vol) in TBS-T) at 20–25°C for 1 h and incubated with primary antibodies diluted in blocking buffer overnight at 4°C. On the next day, sections were washed, and incubated for 1 h with secondary antibodies with 250 nM 4',6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific) for nuclear staining. The immunostained sections were washed and coverslipped with Fluoro-Gel mounting medium (Electron Microscopy Science; Hatfield, PA). Images were acquired with an epifluorescence microscope (Zeiss Axioscope A1; Carl Zeiss AG, Oberkochen, Germany).

Another three non-treated animals were used to determine the distribution of olfactory sensory neurons expressing NQO1 and OMP. Following anesthesia, the animals were perfused transcardially with PBS followed by 4% paraformaldehyde. The head was post-fixed, decalcified in 0.45M EDTA/PBS for 2 weeks, cryo-preserved in 30% sucrose/PBS, and embedded in O.C.T. compound (Sakura Finetek USA). The olfactory tissues were cut on a cryostat into 20µm slices, and stored at –20°C until use. The slices were then immunostained with anti-NQO1 or anti-OMP antibodies as described above.

Statistical analysis

Analysis was performed in a blinded manner for each animal using the transverse nasal sections described by Mery et al (Mery et al., 1994). Based on anatomic considerations, Mery levels were combined for analysis as follows: levels 6 and 7; levels 8 through 10; levels 10–11 and 11; level 15; levels 20 and 20–21; levels 22–23 through 23–24; levels 24–25 through 26; level 27; and levels 29 through 30. Sections rostral to level 6 had no olfactory epithelium. Nasal sections not corresponding closely to a specific Mery level (Mery *et al.*, 1994) were reported as between the closest two levels (e.g. 10–11), and each section was

only considered once for analysis. In addition, the nasal mucosa was then divided into 16 different segments (see Figure 4). Each of the 16 segments was then independently characterized as negative or positive for necrosis and NQO1 expression. A Kappa test (Viera and Garrett, 2005) was then performed to determine the level of agreement between the regions with necrosis and those expressing NQO1. The Cohen's Kappa coefficient of agreement (K) was computed as $K = (p_o - p_e)/(1 - p_e)$. The probability, p_o , is the relative observed agreement corresponding to the proportion of regions which were positive for necrosis and NQO1 expression plus those negative for necrosis and NQO1 expression. The probability, p_e , is the hypothetical probability of chance agreement corresponding to the probabilities of each condition randomly occurring in each category. A coefficient of agreement equal to 1 indicates a perfect agreement between the region expressing NQO1 and necrosis or high flow and necrosis, no agreement would lead to $K < 0$ [see (Viera and Garrett, 2005) for more details].

RESULTS

Period of Exposure

During the 3 hours of exposure, the concentration of H₂S was found to range between 204 and 208 ppm in the inlet port of the chamber. The concentrations in the outlet port were 4 to 7 ppm lower. The flow of gas, which averaged 6 l/min, maintained the fraction of inspired O₂ and CO₂ in the chamber at the same level whether in H₂S or in air, averaging 20.55% and 0.45% respectively. The animals exposed to 200 ppm did not show any signs of discomfort or distress during or after the exposure and their behavior was not different from the animals exposed to air. Their weight increased over the 5 or six-day period of the protocol (3 days exposure and 2 or 3-day recovery) by an average of $2.7 \pm 1\%$, which was similar to the control group.

Effects of H₂S (200 ppm)

Sections of nasal cavity taken from the three air-exposed control rats were unremarkable by histologic examination. In the 8 H₂S-exposed rats, there was bilaterally symmetric acute segmental necrosis limited to the sustentacular cells and sensory neurons of the olfactory epithelium. Necrosis was characterized by loss of apical cilia, nuclear pyknosis, karyolysis and karyorrhexis, and cell swelling and fragmentation with hyaline hypereosinophilic cytoplasm (Fig. 1A and B). Affected regions were sharply demarcated from adjacent unaffected olfactory epithelium, and a distinct spatial pattern was evident. A representation of the regions affected in the five H₂S-exposed animals is shown in Figure 1C. There was no difference between the animals examined at day 2 or day 3 following the end of sulfide exposure. Other regions of the nasal cavity were unaffected.

Examination of the H₂S-exposed rat olfactory epithelium from anterior to posterior showed that necrosis was present in the medial and lateral walls (but not the dorsal reflection) of the dorsal meatus (DM) as well as dorsomedial portions of the first (1E), second (2E) and third ethmoid turbinates (3E), often affecting the dorsal convex reflections of the scrolls (Fig. 2A). The fifth (5E) turbinates were less commonly affected, and rarely were fourth (4E) and sixth (6E) involved.

To correlate the H₂S -induced necrotic region with zonal organization, rat olfactory epithelium was immunohistochemically stained with anti-NQO1 antibody that has been reported to label dorsomedial region, zone 1 (Gussing and Bohm, 2004). Similar to the mouse olfactory epithelium, we found in the control rats that olfactory sensory neurons expressing NQO1-positive were only located in the olfactory epithelium of the dorsal but not of the ventral meatus (Fig. 2B). NQO1 positive neurons were also present on the dorsomedial reflection of the 2E, 3E, 4E, and 5E in a spatially localized manner. In contrast OMP, a marker of olfactory neurons, were distributed over the entire olfactory epithelium. The lack of OMP staining in the ventral and lateral regions of the rostral nasal cavity (Fig. 2C, 2 left panels) reflects the absence of olfactory neurons in these regions, covered by a respiratory epithelium, as previously described by Mery et al. (Mery et al., 1994). Locations of NQO1-positive olfactory sensory neurons overlapped with the regions where OMP-positive olfactory sensory neurons were found in most of zone 1 (Figs. 2B' and 2C'). Of note, since OMP is a cytosolic protein while DAPI staining is only present in the nucleus, dendrites, present at the level of the mucus layer of the OE and axons present at the level of the lamina propria, beneath the OE, show a green without blue staining, but there was no discrepancy at the level of the cell bodies (Fig. 3). Finally, no surrounding sustentacular cells expressed NQO1 or OMP.

Degree of agreement between NQO1 and necrotic regions

As shown in Figures 1 and 2, visual examination of the distribution of sulfide-induced olfactory epithelial necrosis and NQO1 expression shows a striking overlap. To systematically analyze the degree of agreement or overlap between the regions wherein necrosis was present and NQO1 expressed as well as the regions wherein necrosis and NQO1 expression were absent, we analyzed the entire rat olfactory epithelium based on the segmentation described by Mery et al (Mery et al., 1994) (see MATERIALS AND METHODS) and created tables showing the presence/absence of necrosis (Fig. 4A) and NQO1 (Fig. 4B) in each segment. Comparison of two tables showed that the overall level of agreement was extremely high (80.2%), resulting in a coefficient of agreement, K, equal to 0.60 (Kappa test) with a maximum possible unweighted Kappa of 0.97 (Fig. 4C). Discrepancies were however observed in two very well defined regions: first the neurons in the dorsal reflection of the dorsal meatus between the septum medially and first or third ethmoid turbinate laterally consistently expressed NQO1, but were unaffected by hydrogen sulfide. Additionally, the medial reflection of the dorsal scroll of the fifth ethmoid clearly displayed olfactory epithelial necrosis, while NQO1 was not expressed in that area in all the normal rats.

Interestingly, in H₂S-exposed rats, NQO1 expression was virtually abolished even in the regions where necrosis was absent such as in the dorsal reflection of the dorsal meatus between the septum medially and first or third ethmoid turbinate lateral and OMP expression was still present (Fig. 5).

DISCUSSION

We found that the olfactory sensory neurons affected by H₂S toxicity in the rat were localized to the dorsomedial portion of the olfactory epithelium, as previously reported (Brenneman et al., 2000; Brenneman et al., 2002). Brenneman et al. described segmental and bilaterally symmetric necrosis of the olfactory neurons affecting the dorsal meatus, produced by an exposure to 200 ppm H₂S for several hours. Lesions were particularly notable for the sharp demarcations from adjacent unaffected olfactory neurons, suggestive of a cell subtype specific intrinsic sensitivity to H₂S. Our present report shows that the regions sensitive to H₂S are overlapping the regions where NQO1-positive olfactory sensory neurons are located. The regions are typically referred to as olfactory zone 1 in mice. The zone 1 olfactory sensory neurons contain a unique type of neuron expressing the fish-like class I odorant receptors and a group of class II receptors, which project to the dorsomedial part of the olfactory bulb (Tsuboi et al., 2006; Bozza et al., 2009). It is empirically suggested that the scent of predators that induce instinct fear responses in mice is encoded by the olfactory sensory neurons in zone 1 (Kobayakawa et al., 2007; Rosen et al., 2015).

There is a clear boundary between zone 1 and non-zone 1 areas, and NQO1 expression is in fact a defining feature of the olfactory sensory neurons present in zone 1 (Gussing and Bohm, 2004). We have established, for the first time, a similar NQO1 pattern of expression in the rat olfactory epithelium. Zone 1 is also identified as a region negative to OCAM, a transmembrane protein expressed by non-zone1 olfactory sensory neurons in rodents (Yoshihara et al., 1997; Alenius and Bohm, 2003). The OCAM-negative region is present in the macaque monkey and covers larger proportion of the OE than rodents (Horowitz et al., 2014). In addition, NADPH-diaphorase activity, considered as a marker of NQO1 activity, is present in most glomeruli in both macaque and human olfactory bulbs (Alonso et al., 1998; Brinon et al., 1998), making the present findings relevant to human H₂S intoxication and olfaction.

The mechanism for the dorsal distribution of sulfide-induced lesions (Brenneman et al., 2000; Brenneman et al., 2002) has previously been debated (Moulin et al., 2002; Schroeter et al., 2006a). Using a computational fluid dynamics model based on an estimate of nasal “extraction” of H₂S (Schroeter et al., 2006b), the possible influence of the local distribution of H₂S was investigated (Moulin et al., 2002; Schroeter et al., 2006a). Preferential flow patterns are produced in the nasal passage through the different meatuses and were proposed to account for some local variation in sulfide toxicity. However, high flow was also noted in the regions lined by the resistant respiratory epithelium. Further, the existence of a sharp demarcation between normal and adjacent necrotic olfactory epithelium would require an additional mechanism to be involved. Our results suggest that olfactory sensory neurons expressing NQO1 are much more sensitive to the toxicity of H₂S than other olfactory sensory neurons. The sustentacular cells were also affected, while they did not express NQO1, which indicates that other factors mechanistically/biochemically unrelated to the presence of NQO1 but specific to this region increase the susceptibility to H₂S toxicity. A causal link between H₂S induced toxicity and the presence of NQO1 expression, per se, remains an intriguing question that should be clarified in future studies. Finally, dissociation between the disappearance of the expression of NQO1, OMP and necrosis was found in few

instances. This was certainly true for the regions where NQO1 was expressed in baseline condition but where necrosis was absent, i.e. the dorsal reflection of the dorsal meatus between the septum medially and first or third ethmoid turbinate laterally; as illustrated in Figure 5. In these specific regions, NQO1 expression was abolished in intoxicated animals while OMP was still present and the cells were not necrotic. This suggests that NQO1 expression may be affected by H₂S regardless of the presence of a significant necrosis.

In conclusion, the present study shows a significant overlap between olfactory sensory neurons in zone 1 expressing NQO1 and the regions affected by H₂S induced necrosis. While the specific mechanisms accounting for this higher susceptibility of neuronal cells present in this region of the olfactory system remain to be established, the present results suggest that H₂S could be used as a tool to alter neuronal activity in zone 1, a region involved in various behavioral functions in rodents (Kobayakawa et al., 2007; Dewan et al., 2013). The presence of a causal link between NQO1 expression and a higher susceptibility to sulfide toxicity remains an outstanding question with potentially important implications for our understanding of different susceptibility of certain populations of neurons in the central nervous system to H₂S (Sonobe et al., 2015).

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Highlights

- The distribution of NQO1 olfactory neurons in rats recapitulates that in mice
- H₂S toxicity specifically affects NQO1 olfactory neurons expressing
- The present findings may be used to further study the function of these neurons.

Figure 1.

(A) Example of one coronal section of decalcified muzzle of one rat three days after H₂S exposure with discrete foci of severe olfactory epithelial necrosis demarcated within boxed areas. (B) Higher magnifications of turbinate showing a sharp demarcation between healthy epithelium and necrotic olfactory sensory neurons and sustentacular cells with loss of cilia, cytoplasmic hyalinosis and nuclear pyknosis, karyolysis and karyorrhexis. HE stain. (C) Schematic distribution of olfactory epithelium necrosis (in red) for the same coronal section in all five exposed rats (C1 – C5), studied 3 days after exposure, highlighting shared similarities as well as minor differences. The response was identical whether analysis was

done 2 or 3 days after the end of exposure. DM: dorsal meatus; 1E – 6E: first to sixth ethmoids. Scale bars: 1mm in A and 50µm in B1 and B2.

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Figure 2.

(A) Representative distribution of necrotizing lesions affecting the olfactory epithelium of H_2S exposed rats analyzed 2 days after the exposure across different sections. The foci of olfactory epithelial necrosis are demarcated with red lines. H&E stain. (B, C) NQO1 (B) and OMP (C) expression in the rat olfactory epithelium. Coronal sections of rat nasal cavity immunostained with anti-NQO1 or anti-OMP (green) antibodies. All nuclei were counterstained with DAPI (blue). NQO1-positive olfactory sensory neurons are located in the dorsomedial meatus (indicated with yellow lines), but not in ventral meatus. Insets (B' and C') show the expression of both NQO1 (B') and OMP (C') by olfactory sensory neurons in the medial wall of DM. Scale bars, 1mm in A, B, and C, 50 μ m in B' and C'.

Figure 3.

(A) Coronal section, displayed in figure 2C slice #4, is shown on separate pictures immunostained with anti-OMP (green) antibody and DAPI (blue). (B) Magnification of OE shown in the red box in (A). As only dendrites are present in the surface of the olfactory epithelium (Mucus layer (ML)), only OMP (which is a cytosolic protein, green color) can be found. Therefore, absence of DAPI (nuclei) in the ML is to be expected. Similarly, in the Lamina propria (LP), beneath the OE, the green signal is originating from fasciculated bundles of OSN axons. Therefore, in this region, no overlap between blue (DAPI) signal and green signal in the LP is expected (asterisks). Inset (B') shows a magnified image of the red-boxed area in (B). All cell bodies showing OMP staining in the OE have also DAPI-positive

nuclei (plus signs). The green signal is surrounding the blue signal as the distribution of the dyes reflects the different localization of OMP (cytosolic) and DAPI (nuclei). Scale bars, 1mm in A, 100 μ m in B, 10 μ m in B'.

Figure 4.

(A) Regions displaying necrosis (red circles) or intact (black circles) after H₂S exposure. (B) NQO1-positive and -negative regions were shown in red and black circles, respectively. (C) Segments that showed whether necrosis+/NQO1+ or necrosis-/NQO1- are shown in red circles (Agreement). While segments that showed disagreement, necrosis-/NQO1+ and necrosis+/NQO1-, were shown in blue and yellow circles, respectively. The segments of rat olfactory epithelium were determined based on the description in Mery et al. (see MATERIALS AND METHODS). The following regions were studied: DM-MW: Medial wall of DM (septum); DM-DR: Dorsal reflection of DM; DM-LW: Lateral wall of DM

including medial aspect of 1E or 3E where appropriate; 1E-R: 1E except lateral wall of DM; 2E-M: Medial convex reflection of s2E; 2E-R: 2E except medial convex reflection; 3E-DMD: Dorsomedial reflection of dorsal scroll of 3E; 3E-VD: Ventral reflection of dorsal scroll of 3E; 3E-DMV: Dorsomedial reflection of ventral scroll of 3E; 3E-R: 3E excluding sites described above; 4E-DM: Dorsomedial reflection of 4E; 4E-R: 4E except dorsomedial reflection; 5E-DMD: Dorsomedial reflection of dorsal scroll of 5E; 5E-R: 5E except dorsomedial reflection of dorsal scroll; 6E-DD: Dorsal reflection of dorsal scroll of 6E; 6E-R: 6E excluding 6E-DD.

Figure 5.

(A–D) Loss of NQO1-positive olfactory sensory neurons after H₂S exposure Expression of NQO1 (A, B) and OMP (C, D) in the dorsal meatus of rat olfactory epithelium was immunohistochemically detected three days after the last exposure to air (A, C) or H₂S (B, D). NQO1 expression is virtually abolished in the H₂S-exposed rat olfactory epithelium, while immunoreactivity to OMP still remains. Not that this region was one of few regions expressing NQO1 but with no necrosis during sulfide exposure. Scale bars, 200μm.