Sensory Detection and Responses to Toxic Gases

Mechanisms, Health Effects, and Countermeasures

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The inhalation of reactive gases and vapors can lead to severe damage of the airways and lung, compromising the function of the respiratory system. Exposures to oxidizing, electrophilic, acidic, or basic gases frequently occur in occupational and ambient environments. Corrosive gases and vapors such as chlorine, phosgene, and chloropicrin were used as warfare agents and in terrorist acts. Chemical airway exposures are detected by the olfactory, gustatory, and nociceptive sensory systems that initiate protective physiological and behavioral responses. This review focuses on the role of airway nociceptive sensory neurons in chemical sensing and discusses the recent discovery of neuronal receptors for reactive chemicals. Using physiological, imaging, and genetic approaches, Transient Receptor Potential (TRP) ion channels in sensory neurons were shown to respond to a wide range of noxious chemical stimuli, initiating pain, respiratory depression, cough, glandular secretions, and other protective responses. TRPA1, a TRP ion channel expressed in chemosensory C-fibers, is activated by almost all oxidizing and electrophilic chemicals, including chlorine, acrolein, tear gas agents, and methyl isocyanate, the highly noxious chemical released in the Bhopal disaster. Chemicals likely activate TRPA1 through covalent protein modification. Animal studies using TRPA1 antagonists or TRPA1-deficient mice confirmed the role of TRPA1 in chemically induced respiratory reflexes, pain, and inflammation *in vivo*. New research shows that sensory neurons are not merely passive sensors of chemical exposures. Sensory channels such as TRPA1 are essential for maintenance of airway inflammation in asthma and may contribute to the progression of airway injury following high-level chemical exposures.

Keywords: chemosensation TRPA1 channel; irritants; airway obstruction; chemical injury

Toxic inhalation hazards (TIHs) are noxious gases and vapors that are harmful and often deadly when inhaled. Specific sensory receptors in the airways sense the presence of these chemicals and initiate conscious, involuntary, autonomic, inflammatory, and other responses to them (1). Some inhaled toxicants have their primary actions in tissues outside of the lungs or hinder oxygen from reaching the alveolar sacs. Usually the only noticeable sensations by these toxicants are their smell and taste, or they are not detected at all and are only recognizable by the signs and symptoms of their toxicity (2). Most TIHs are reactive chemical gases and vapors that can severely change the structures of the proteins, lipids, DNA, and other biomolecules, resulting in a loss of protein function, DNA

mutations, and necrosis of airway epithelial cells (3, 4). The body defends itself against these “reactive-TIHs” with the tissue barriers presented by hair, skin, and mucous membranes, as well as by extracellular fibers, fluids, membrane, cytosolic components, and glandular secretions containing high concentrations of antioxidants, buffers, and other compounds that sequester and neutralize reactive chemicals. These include uric acid, glutathione, ascorbic acid, carotenes, lipoic acid, tocopherol, ubiquinol, ammonia, carbonic acid, phosphate, and enzymes. In certain incidences these enzymes produce more toxic metabolites (5–7). In severe exposures these protective chemicals are saturated and the reactive chemicals react with lung tissues, damaging lung and its delicate alveolar sacs (8). The airways have specialized mechanisms to protect alveoli from damage. The peripheral sensory neurons of the airways express specific acid-sensitive ion channel (ASIC) and TRP cation channels that are directly activated by reactive chemicals. The activation of these ion channels triggers signaling to the brain to perceive pain and to initiate involuntary, autonomic and motor reflexes of the glands and muscles of the airways and lungs (9–14). The activated channels also induce the sensory neuronal peripheral endings to release proinflammatory factors near the site of contact with the reactive TIH (15).

At trace levels, the reactive TIHs are perceived as pungent and irritating, and induce sneezing, coughing, mucus secretion, upper airway inflammation, and tearing. At higher levels, these sensory neuron-mediated sensations and responses progress to incapacitating pain, uncontrollable coughing, profuse lacrimation, and resistance to airflow by bronchospasm, mucus hypersecretion, and upper and lower airway inflammation (pneumonitis) (10, 16–21). These responses neutralize, hinder, and expel toxic materials, limiting damage to the delicate alveolar sacs. However, continued exposures can lead to exaggerated responses that compromise respiratory function (20, 22). Reactive TIHs can compound pre-existing conditions. For example, they are notorious for triggering attacks in individuals with asthma (irritant-induced asthma) (23). The continual respiratory responses might be involved in the development of chronic airway diseases, including bronchitis and occupational asthma, and reactive airway dysfunction syndrome (20–22, 24–29). Exaggerated sensory neuronal responses may contribute to the pulmonary edema and adult respiratory distress syndrome (ARDS) seen with high concentration exposure of TIHs (30, 31). Therefore, not only must the airways be able to immediately detect the presence of reactive TIHs and initiate the appropriate responses to prevent exposure to the alveolar sacs, they must also deactivate this in a controlled manner to allow for normal breathing and blood flow to occur once the threat has disappeared.

Injury and inflammation alter sensory neurons to become hyperactive or hypersensitive to noxious stimuli. This phenomenon has been thoroughly characterized in pain conditions such as migraine and chronic inflammatory or neuropathic pain. It would seem likely that similar mechanisms occur in the airway sensory innervation (32–34). The necrotic and damaged cells of the airway epithelium release signaling molecules, such as

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DNA, ATP, adenosine, uric acid, other small molecules, peptides, peptidase products, lipids, and lipid-oxidation products. These molecules act as warning signals to the nearby tissues, and initiate and modulate inflammatory responses by the surrounding cells, especially the front line immune cells (4). These signaling molecules, cytokines, and other inflammatory mediators released during an airway injury modulate the sensitivity and expression of sensory neuronal TIH receptors (35, 36) (see Table 2 for TRPA1 modulators). This could result in chronic or hypersensitive neurogenic inflammatory responses in the lungs and airways and possibly lead to chronic inflammatory lung diseases or hypersensitivity conditions (37).

Currently, the therapy for acute exposures to reactive TIHs is their removal by dilution, washing, and chemical neutralization, the treatment of pain and inflammation with anti-inflammatory medications and analgesics, and stabilization of the airways with bronchodilators (38). Morbidity from high-level exposures to reactive TIHs can occur after the initial structural damage. Sensory neuron-mediated inflammation may have role in pulmonary edema, pneumonitis, and other complications of ARDS implicated in fatalities from high-level exposures (30). Occupational asthma can develop from reactive TIH exposure in workplace settings, and this is usually treated by counteracting the symptoms of airway inflammation and smooth muscle constriction. Antagonists blocking TIH receptors and or modulators of these receptors may provide novel therapeutics to alleviate the irritation and inflammation resulting from acute exposures. This may in turn abate the inflammation and pulmonary edema caused by high-level exposures and treat the chronic lung and airway diseases associated with reactive chemical inhalation.

INTRODUCTION OF TOXIC INHALATION HAZARDS

Noxious gases and other inhalation toxins are widely used and produced in the modern industrial world. The ease and low cost of manufacturing of these chemicals, plus their high toxicity and relatively short half-life, make them very beneficial as fumigants, nematocides, fungicides, disinfectants, and sanitizers in agriculture, pest control, disinfection, cleaning, and water purification industries. Many TIHs are important reactants in metallurgy and in the organic synthesis of plastics, pharmaceuticals, semiconductors, and other materials. Copious amounts of chlorine gas and other TIHs are used for bleaching and chemical synthesis, and large quantities of ammonia and nitric acid are used for fertilizer production (2, 39). TIHs are created as by-products of water purification, metal smelting, and combustion, as well as by the interaction of combustion by-products with ultraviolet light and oxygen in the air or water (40). This includes oxides and other toxins produced in welding and numerous other air pollutants from industry, automobiles, smelters, forest fires, cigarette smoke, and volcanoes (2). Workers in industrial, custodial, agricultural, and other occupations with low but frequent exposures to reactive TIHs, as well as welders, smokers, and people in areas of frequent air pollution, often suffer from the chronic airway and lung diseases (41–43).

To maintain a supply for their use, large volumes of TIHs are transported and stored, sometimes near large population centers. Unfortunately, accidental leaks from industrial facilities and transportation vehicles do occur. The resulting high-concentration exposures to TIH have caused immediate large-scale evacuations, fatalities, and severe injuries of the lungs and other tissues in the survivors (44). The most infamous accident involving TIHs was the tragedy of Bhopal, India, where thousands of residents died from exposure to methyl isocyanate (45). High exposures of TIHs were used in chemical warfare, especially in World War I, and

have since been outlawed by international treaties. Unfortunately, rogue military regimes and terrorists have used gaseous warfare agents to attack and indiscriminately kill and injure civilian populations (46, 47).

Expectantly, state, federal, and international law and agencies tightly regulate the use, storage, transportation, emissions, and disposal of TIHs, and the exposure levels allowed in the environment and the workplace. The executive agencies of the United States concerned with these regulations have classified and described the various TIHs. This information is accessible through the Material Safety Data Sheets, the websites of the individual agencies, and the National Library of Medicine Toxicological Data Network (ToxNet) website (<http://toxnet.nlm.nih.gov>).

The occupational health database, Haz-Map (<http://hazmap.nlm.nih.gov>), within ToxNet has classified about 220 chemicals as “Toxic Gases and Vapors” (this group is not all the TIH; for instance, the database has ~450 chemicals that can induce pneumonitis). In Table 1, these “Toxic Gases and Vapors” are loosely categorized by their chemistry and the mechanism of their toxicity (2). Category one includes chemicals that target the cholinergic systems (acetylcholine antagonists and acetylcholine esterase inhibitors). Category 2 includes chemicals that deplete or hinder oxygen from reaching the alveoli and thereby asphyxiate (simple asphyxiates). Category 3 included chemicals that target metal ions in vital enzymes, cytochrome c oxidase, hemoglobin, and other metalloproteins (cyanides, aliphatic nitriles, metalloid hydrides, and carbon monoxide). The cyanide anion is a nucleophile that readily forms nitriles. This reaction is readily reversible in biological systems for most aliphatic nitriles, thereby producing cyanide, but less reversible for aromatic nitriles (48, 49). Category 4 includes chemicals that induce severe blistering, including blistering and damage to the airways (arsines and mustard gases). Some of these vesicants are only detectable by their odor and the blistering effects do not occur until hours after exposure. Category 5 includes chemicals that react indiscriminately with most biological molecules. These can be further categorized by the type of chemical reactant (acids, corrosive gases, oxidants, reducers, electrophiles, and nucleophiles) and the type of chemical (isocyanate, halogen). Some chemicals belong to multiple subcategories, and members of a category may differ in their chemical properties but react in a manner similar to other members of the category. For example, often acid halides will produce an alkylation of a protein and a strong acid, both contributing to the toxicity. Members of the reactive chemical subcategories usually induce similar symptoms (irritation, pneumonitis, and pulmonary edema). Only a few sensory neuron ion channels are responsible for detecting most of the TIHs and initiating many of their symptoms. TIHs activate these channels by forming chemical reactions with them. Therefore, TIHs that form similar chemical reactions will activate the same channel and have similar symptoms, even if the chemical structures are different.

MECHANISMS

Olfactory and Gustatory Chemoreceptors

The major sensations induced by exogenous chemicals are smell, detected by olfactory neurons; taste, detected by the taste receptor cells in the taste buds; and pungency/irritation/pain that are mediated by peripheral sensory neurons (50). Most TIHs are associated with an odor and many elicit a taste sensation, such as the sour taste of acids and the bitter taste of cyanide. In addition to being an important aspect of consciously identifying TIHs, odors and tastes may have a role in initiating

peripheral physiological responses. This is probably a more important mechanism in animals with more sophisticated olfactory systems than humans, such as rodents and certain insects (51). The odors of the volatile organic sulfur compounds and some other TIHs are innately repulsive and nauseating (52). However, this repulsive sensation can desensitize with continued exposure, and toxic exposures have occurred without victims becoming aware of the presence of the sulfide compound (53). Certain TIHs have an agreeable smell and elicit no acute noxious sensations, so their avoidance behavior has to be learned. For example, the faint and agreeable odor of certain nerve gases is the only physiological mechanism to recognize them until the hypercholinergic effects occur (54). Carbon monoxide, some simple asphyxiates, and the chemical weapon quinuclidinyl benzilate (BZ) are odorless and can go undetected until victims show effects from their toxicity (2).

The chemoreceptors for odors are specific ligand-gated G protein-coupled receptors (GPCRs) on olfactory neurons that stimulate adenylate cyclase to synthesize cAMP via a G protein called G_{olf} . Insects also express ion channel odorant receptors (55). The receptor for bitter-tasting chemicals is the GPCR TAS2Rn, which is coupled with the G protein gustducin. Interestingly, these receptors are also expressed in the motile cilia of the airway epithelium. The α -subunit of gustducin facilitates the activity of cAMP phosphodiesterase by binding inhibitory subunits and thereby decreasing intracellular cAMP levels, while the β -subunit activates PLC $\beta 2$ and subsequently increases intracellular Ca^{2+} . The rise in Ca^{2+} gates the monovalent TRP ion channel TRPM5. This produces a bitter taste sensation mediated by the taste buds and increases the activity of cilia to expel mucous-bound-toxin up and out of the lung (56–58).

The acidity-invoked sour sensation is not completely understood at the receptor level. It is likely due to H^+ and H_3O^+ cations activating specific ion channels. Different acid receptor proteins have been proposed, including members of the ASICs (a sub-family of the amiloride-sensitive Na^+ channels), hyperpolarization-activated cyclic nucleotide-gated K^+ channels (HCNs), 2-pore domain K^+ leak conductance channels, and the TRP ion channel TRPP3 and its partner PKD1L3 (59). It is possible that ASIC channels are directly activated by low pH, while TRPP3 and PKD1L3 are primed by acidity, and subsequently activated by return to physiological pH (60, 61).

Sensory Neuron Chemoreceptors

Most TIHs (80–90% of the TIHs in Haz-Map) are reactive chemicals and produce similar sensations of a pungent “burning” irritation and pain by activating chemoreceptor sensory neurons. The airway mucosal epithelium is densely innervated with projections from the peripheral nerves of the somatic sensory system, including two nerve fiber types involved in chemical nociception (62). These fibers are anatomically distinguishable from each other by their diameter and degree of myelination. A- δ fibers have a slightly larger diameter and are encased in thin myelin sheaths, while the C-fiber neurons are unmyelinated with smaller diameters. Activation of A- δ fibers produces a fast, sharp pain sensation, while the slow velocity C-fiber activation induces a slow “burning” pain. In the respiratory system, these fibers correspond through the trigeminal or vagus nerve with brainstem nuclei to coordinate sensations. This results in reflexes and responses to deter further inhalation, expel toxicants by coughing and sneezing, dilute and neutralize reactive toxins by parasympathetic glandular secretions, and alter breathing patterns and other brain-mediated airway responses to reactive TIHs (10, 16–21). In rodents, TIHs activation of sensory neurons induces a decrease in respiratory rate by increasing the pause between individual breaths. This is

used as a common model to measure and determine respiratory irritation by TIHs (16, 18, 63).

Reactive TIHs also induce peripheral endings of C-fiber neurons to secrete peptides and other molecules at and near the site of contact, such as Substance P (SP) and calcitonin gene-related peptide (CGRP). SP preferentially binds to the tachykinin receptor NK1 receptors, while CGRP binds to calcitonin receptor-like receptor (CRLR) coupled with the receptor activity modifying protein (RAMP1 or 3). NK1 receptors are GPCRs coupled to the Gq subunit, which activates PLC and increases intracellular Ca^{2+} . CGRP receptors (CRLR+RAMP) are coupled to the Gs subunit, which activates adenylate cyclase, increasing intracellular cAMP. These peptide receptors show partially overlapping expression patterns. In certain instances their actions are synergistic, while in other instances they counteract each other (34, 64, 65). Extensive studies on SP and CGRP have shown that they are predominantly proinflammatory factors that increase bronchoconstriction, immune cell infiltration, vasodilation, mast cell degranulation, plasma extravasation, and other local inflammatory responses (34). The acute pneumonitis from certain reactive TIHs most likely has a neurogenic inflammatory component. Possibly, the pulmonary edema resulting from reactive TIH exposure also has a sensory neuronal component. Substance P and CGRP have been proposed to be involved in the pathology of allergic asthma (66, 67). Occupational asthma and reactive airway dysfunction syndrome may also have a neurogenic inflammatory component (68).

Acid Sensing: ASICs

Acidic TIHs stimulate both A- δ and C-fiber sensory neurons. This results in acidic TIH induction of a sharp, immediate pungent pain and reflex-like breath withdrawal as well as burning pain, pneumonitis, coughing, mucus secretion, and other responses (69–71). C-fibers appear to have at least two distinct electrophysiological currents sensitive to acidic pH, suggesting at least two distinct ion channel populations responding to acidic stimuli. An influx of Na^+ occurs at pH levels slightly below the homeostatic level that rapidly inactivates and a mixed Ca^{2+}/Na^+ influx occurs at more acidic conditions. Various combinations of splice-variants of the three members of the ASIC (ASIC1a, ASIC1b, ASIC2a, ASIC2b, and ASIC3) family of ion channels appear to be responsible for the first currents. These channels are expressed in both A- δ and C-fiber sensory neurons and show similar electrophysiological properties in heterologous expression systems, with conducting Na^+ currents induced between pH 6 and 7 that rapidly inactivate. Different combinations of subunits and splice variants of the three ASIC genes may explain the observed differences in kinetics and pH sensitivity in native neurons (59).

Acid-Sensing Ion Channels: TRPV1

At lower pH (~ 5) the nonspecific cation channel TRPV1 is activated in sensory neurons (72). While the various heteromeric combinations of ASIC-mediated acid-sensitive channels are ubiquitous in both chemosensory fiber types, the splice-variant and subtype distribution of ASICs have some specificity. TRPV1-like acid-sensitive currents are primarily observed in unmyelinated C-fiber neurons (59). These currents correlate with TRPV1 expression and the currents are not observed in the sensory neurons of mice devoid of TRPV1. While TRPV1 is activated at relatively low pH, inflammatory mediators and injury signals can augment the sensitivity of TRPV1 to acidity, and acidity can in turn hypersensitize TRPV1 (73). While it is unlikely that the pH in the lung would drop low enough to

TABLE 1. TOXIC INHALATION HAZARDS AND THEIR MECHANISMS OF INJURY

Toxic Gas or Vapor	No. of TIHs	Sensory Recognition			Toxicity			
		Irritate	Odor	Receptor	Pneu monitis	Edema	Signs and Symptoms	Mechanism of Toxicity
Cholinergic toxins	7	No	None to fruity	M1 and M2	No	No	Miosis (BZ opposite)	Parasympathetic
Simple asphyxiants	15	No	No	Carotid	No	No	Suffocation	Asphyxia
Target Fe cations								
Carbon monoxide	1	No	No		No	No	Lassitude	Binds to O ₂ site on hemoglobin
Cyanide salts, halo-, HCN	12	Some	Bitter almond				Pain, lassitude	Electron transport
Aliphatic nitriles	34	Yes	Varies		Yes	Yes	Pain, lassitude	Electron transport
Metalloid hydrides	8	Yes	Varies		Yes	Yes	Pain, edema, tremors	Heme, CYP, and other sites, reducers
Vesicants								
Mustard gases	3	No	Fish, garlic, musty		Yes	Yes	Late onset blistering	Perhaps onium ions
(Nucleophile/ Electrophile)								
Arsines-chemical weapons	5	Yes	Agreeable to nausea		Yes	Yes	Pain, vomit, blisters	
Electrophiles								
Chloroformates and similar acid halides	36	Yes	Pungent	AISC TRPV1	Yes	Yes	Pain,	Alkylation of biomolecules
Phosgenes	3	Only Oxime	Cut hay, fruity, pungent		Yes	Yes	Pain for oxime, respiratory distress	Alkylation of biomolecules
Aromatic nitriles	13	Yes	Varies	TRPA1			Pain	Alkylation of biomolecules
Isocyanates and O- and Cl ⁻ alkenes	17	Yes	Pungent	TRPA1	Yes	Yes	Pain	Alkylation of biomolecules
Oxidizers								
Oxides, halides, halogens	16	Yes	Pungent	TRPA1	Yes	Yes	Pain	Alkylation of biomolecules
Chlorosilanes	11	Yes	Pungent		Yes	Yes	Pain	Alkylation of biomolecules
Nitroxides	5	Yes	Pungent	TRPA1	Yes	Yes	Pain	Alkylation of biomolecules
Reducers								
Hydrazines	3	Yes	Fish NH ₄		Yes			Hemolytic
Hydrogen sulfide and thiols (weak nucleophiles)	4	Yes	Rotten eggs		Yes	Yes	Vomiting	Asphyxiant
Corrosive gases	12	Yes	Pungent	TRPA1				React w/biomolecules
Acids/acids in H ₂ O	18	Yes	Acrid	AISC TRPV1	Yes	Yes		React w/biomolecules

Definition of abbreviation: TIH = toxic inhalation hazard.

The rows of the table are a loose subjugation of those chemicals considered "Toxic Gases and Vapors" by the Haz-Map (<http://hazmap.nlm.nih.gov>) website based on their toxicity and chemistry. The first column identifies the toxicants group, the second is the number of the almost 250 chemicals grouped into this classification, next are sensations induced (respiratory irritation and odor), followed by a column of sensory receptor proteins/systems activated by these toxicants, and the last columns are the toxic effects and symptoms.

activate TRPV1 during inhalation of a TIH, repeated inhalation or underlying inflammation may facilitate TRPV1 to become hypersensitive and therefore triggered by an exposure to a gaseous acid (73–80). For instance, TRPV1 is sensitized by the potent proinflammatory signal, bradykinin (81).

TRPV1 is well established to have a crucial role in the nociception of heat. It is the site of action for the active ingredient in chili peppers, capsaicin. There are symptom similarities between high-level exposures to capsaicin as pepper spray (OC tear gas) and exposures to reactive chemical TIHs. However, studies of TRPV1 gene-deleted mice still show normal irritant responses to TIHs mediated by sensory neurons (82).

Most C fibers express either TRPV1 (80–90%) or the cold activated ion channel, TRPM8 (8%). TRPM8 is sensitive to cool temperatures and is at least partially responsible for the sensation elicited by cool temperatures and by menthol and related compounds. Similar to TRPP3 and TRPM5, these proteins belong to the Transient Receptor Potential (TRP) superfamily of cation channels. TRP proteins are believed to form tetrameric cation channels in a manner similar to the voltage-gated K⁺ channels, with six transmembrane spans and an ion selectivity filter-pore between the fifth and the sixth span. Both the amino and carboxyl terminal spans are cytosolic. Most TRP channels conduct and are regulated by calcium ions. Predictably, their physiological functions often correlate with

the dynamic roles that calcium performs in cells, organisms, and disease, especially in sensing and signaling (83).

Oxidant and Electrophile Sensing: TRPA1

TRPA1, a nonspecific cation channel expressed in 30–40% of the TRPV1-expressing C-fiber neurons, is directly activated by volatile reactive organic electrophiles or oxidative agents (~40% TIHs on Haz-Map site shown in Table 1) (84). This has been demonstrated *in vitro* and *in vivo* in numerous publications using TRPA1-gene deleted mice or treatments with TRPA1-antagonists. Table 2 provides a relatively inclusive list of all the TIHs and related chemicals found thus far to activate TRPA1. However, TRPA1's role in the airway responses to many of these reactive chemical TIHs has yet to be characterized. The most potent and specific TRPA1 agonists characterized so far are the CS-, CR-, and CN-tear gases (riot control agents) (85, 86). These agents appear to target TRPA1 to induce their intended effects of incapacitating pain, glandular secretions, coughing, acute neurogenic inflammation, and other symptoms associated with exposures to reactive chemical TIHs.

Just before the first putative transmembrane span of TRPA1 a short protein moiety is located containing three cysteine residues and a lysine residue. These amino acid residues form a "binding site" for the protein modifying TIHs. It is believed that covalent modification of these residues leads to activation

TABLE 2. TOXIC INHALATION HAZARDS ACTIVATING THE SENSORY NEURONAL ION CHANNEL TRPA1.

Type of Chemical	Toxicant	Sensory Neuron	Ex Vivo, In Vivo Experiments	Trpa1 ^{-/-} Antag	Ref. No.
Allergen	Ovalbumin		MeCh Resistance	Both	(97)
Corrosive gas	Ammonium chloride	DRG	Pain	Trpa1 ^{-/-}	(93)
Electrophile	Oximes			AP18	(104)
Electrophile	Trans-2-pentenal	TG		Trpa1 ^{-/-}	(105)
Electrophile	Toluene diisocyanate	TG	Plethysmography	Trpa1 ^{-/-}	(106)
Electrophile	Methyl isocyanate	DRG		Trpa1 ^{-/-}	(86)
Electrophile	Hexamethylene diisocyanate	DRG	Pain	Both	(86)
Electrophile	Benzyl bromide	DRG			(86)
Electrophile	Bromoacetone	DRG	Pain	Trpa1 ^{-/-}	(86)
Electrophile	Crotonaldehyde	JG	Bronchus	Both	(95)
Electrophile	Acrolein	JG, TG	Bronchus, pain, heat	Both	(95, 107)
Electrophile	Allylisothiocyanate	DRG, TG	Pain, artery	Both	(108)
Electrophile	Methylvinylketone				(109)
Electrophile	Methacrolein				(109)
Electrophile tear gas	Chloroacetophenone	DRG	Pain	Both	(85, 86)
Electrophile tear gas	Chlorobenzalmononitrile	DRG	Pain	Both	(85, 86)
Electrophile tear gas	Dibenzoxazepine	DRG	Pain	Both	(85, 86)
Electrophile fumigant	Chloropicrin				(86)
Electrophile air pollutant	Cigarette smoke extract	JG	Bronchus	Both	(95)
Aldehyde	Acetaldehyde	TG	Pain	Camphor	(110)
Aldehyde	Formalin	DRG	Pain	HC	(101)
Halide amine	Iodoacetamide				(88)
Oxidant	Ozone	Vagal gang	Lung	Trpa1 ^{-/-}	(111)
Oxidant	Chlorine	DRG/TG	Plethysmography	Trpa1 ^{-/-}	(84)
Oxidant	Hydrogen peroxide	DRG/TG	Plethys/pain	Trpa1 ^{-/-}	(84, 112, 113)
Modulator	Substance P		Scratching		(114)
Modulator	Nitric oxide	DRG	Pain, heat	Trpa1 ^{-/-}	(115, 116)
Modulator	GDGF and BDGF	DRG			(117)
Modulator	Bradykinin	Vagal/nodose	Esophy nerve	Trpa1 ^{-/-}	(11, 96, 107)
Modulator	Ca ²⁺ intracellular				(12, 13, 118)
Modulator	Enzyme/PAR2	DRG			(14)
Modulator	NGF	TG			(119)
Modulator	Artemin	DRG			(120)

Definition of abbreviations: BDGF = brain-derived growth factor; DRG = dorsal root ganglia; GDGF = glioma-derived growth factor; JG = jugular ganglia; TG = trigeminal ganglia.

This table displays the various toxic inhalation hazards found to activate the sensory neuron ion channel, TRPA1. The first column is for the chemistry of the toxicant, the next is the toxicant identified to be a TRPA1 agonist, followed by sensory neurons studied, how the effect of the toxicant on this channel was examined *in vivo* and *ex vivo*, and whether TRPA1 was involvement in the responses with an antagonist to TRPA1 or mutant mice lacking a functional *trpa1* gene. The last column provides a reference for the study that made these findings.

of the channel, although additional residues have been shown to also be involved (87–89). Figure 1 shows the likely modification of cysteine residues by certain types of TIH molecules that are electrophiles or oxidants. As mentioned, some of these reactive organic molecules have yet to be examined for activity on TRPA1 (90–92). Most likely, if the molecule can form covalent bonds with the cysteine residues of glutathione, then it will also react with the cysteines in the “covalent modification binding site” and thereby activate TRPA1 and the C-type sensory neuron. Interestingly, ammonia also activates TRPA1, probably through alkalization of the cytosolic spans (93).

Similar to many TRP channels, TRPA1 conducts both divalent and monovalent cations. TRPA1-carried cations depolarize the membrane, thereby activating voltage-gated Na⁺ and Ca²⁺ channels to propagate action potentials and consequently signal the spinal and brainstem nerves to initiate reflexes and autonomic and other protective responses of the airways and the lungs (94). In TRPA1-deficient (*Trpa1*^{-/-}) mice or mice treated with a TRPA1-antagonist, pain, neurogenic inflammation, bronchial constriction, and respiratory depression in response to reactive chemicals are absent or abated (*see* Table 2, second to last row). Activation of TRPA1 also results in the secretion of proinflammatory factors such as Substance P and CGRP (95). Neuronal peptide release is thought to be triggered by the influx of Ca²⁺ into the airway nerve ending, either directly through TRPA1 or by TRPA1-triggered voltage-gated Ca²⁺ channels. However, other mech-

anisms may be involved. About half of TRPA1's amino acid sequence is dedicated to a span of about 17 ankyrin repeat domains on the amino terminal span that may interact with signaling complexes and the vesicle release machinery.

Some of the necrotic cell-derived factors and mediators released as a consequence of chemical injury and inflammation modulate the sensitivity and activity of TRPA1 (*see* Table 2). Hyperactive or hypersensitive TRPA1 channels may contribute to chronic bronchitis, occupational asthma, and other inflammatory lung diseases associated with exposures to reactive TIHs (96). TRPA1 is crucially involved in allergenic respiratory sensitivity to ovalbumin, an inducer of allergic asthma in animal models (97). These findings show that airway sensory neurons actively modulate the local immune response to allergens.

Phosgene, Ozone, and Nitrogen Dioxide

The relatively hydrophobic reactive chemicals, phosgene, ozone, and nitrogen dioxide, do not readily diffuse through the mucus and epithelium to reach underlying sensory nerve endings (2). Therefore, they are usually not detected by sensory neurons and do not trigger defensive responses, thereby permitting the toxicants to penetrate more deeply into the lungs to the delicate alveolar sacs. The inability of the chemosensory system to detect these hydrophobic TIHs may compound the damage these chemicals inflict in the respiratory system. An additional factor to their toxicity is that hydrophobic TIHs do not readily interact

Acid Halides **Nitriles** **Phosgene** **Unsaturated Aldehydes** **Isocyanates** **Oxidizing Agents**

Figure 1. Chemical structure drawings representing the possible interaction of a cysteine at the TRPA1 “covalent modification binding site” and a toxic inhalation hazard chemical. Each of the reactions shown is with a chemical known to activate TRPA1 or that induces symptoms and effects similar to TRPA1 agonists. These chemicals tend to be electrophiles and oxidants.

with the aqueous compounds in the airway fluids that sequester and neutralize reactive chemicals.

HEALTH EFFECTS OF TIHS AND COUNTERMEASURES

The TIHs can be loosely grouped into those that are reactive chemicals and those that are not. The major sensations to “nonreactive TIHs” are smell and taste or a lack of any sensation. The sense of smell is important to recognize the danger, so the appropriate protective measures can be made. In addition to having an odor/taste associated with them, most of the “reactive TIHs” activate specific receptors on the somatosensory peripheral nerves of the airways. The receptors for the “reactive TIHs” may become hyperactive, hypersensitive, or overexpressed. As summarized above, these receptors are cation channels that stimulate the sensory nerves to initiate a variety of physiological responses, including local neurogenic inflammation and reflex-mediated responses, such as bronchospasms, mucus hypersecretion, coughing, and central sensations such as pain. Therefore, hyperactive or hypersensitive channels may cause continuous or hypersensitive inflammation, airway

resistance, coughing, and pain, and possibly contribute to chronic inflammatory lung diseases. Targeting these ion channels may not only alleviate the acute effects from exposure to a reactive TIH, but also have a role in therapy for the lung diseases and syndromes associated with reactive TIHs.

The sensory neuronal receptors for the “reactive TIHs” have been identified. Slight acidity activates ASIC Na^+ channels, while stronger acidity activates TRPV1 $\text{Ca}^{2+}/\text{Na}^+$ conducting channels. The ASIC channel antagonist, amiloride, might be effective in treating people exposed to acidic TIHs, and more specific antagonists have been developed (98). In the last few years, the pharmaceutical industry has produced several potent TRPV1 antagonists with hopes of treating ailments such as chronic cough, chronic pain, and arthritis. However, the role of TRPV1 in heat sensitivity and its apparent function in the regulation of body core temperature caused potentially detrimental side effects (99, 100). Topical and acute applications, such as an aerosol spray of TRPV1 antagonists, may minimize these side effects, while being efficacious in treating airway irritation and inflammation. TIHs that are electrophiles and oxidants activate the $\text{Ca}^{2+}/\text{Na}^+$ channel TRPA1. Antagonists to TRPA1

have been discovered, but are in the early phases of research and development and are currently not ready for clinical use (101–103). Rodents treated with these antagonists have shown reduced nociception and inflammatory peptide release in response to reactive TIHs (see Table 2).

Inflammatory mediators resulting from tissue injury, immune challenge, or other sources can modulate TRPA1, TRPV1, and other sensory neuron ion channels. Current therapies for reactive TIH-induced airway irritation and inflammation target these mediators, and include methyl xanthines as well as steroidal and nonsteroidal antiinflammatory drugs. Perhaps part of these medications' therapeutic effect is to diminish the modulation and facilitation of reactive TIH receptors.

Activation of TRPA1, TRPV1, AISCs, and other C-fiber ion channels can induce the release of proinflammatory peptides, such as SP and CGRP. These peptides mediate many of the sensory neuron inflammatory effects and may have a role in the asthmatic-like symptoms seen with frequent low-level exposures to TIHs. CGRP antagonists are used in treating migraine headaches and may also prove beneficial to decrease neurogenic inflammation in the airways.

In summary, most toxic gases and vapors are detected by olfactory neurons and taste receptor cells. Those that are reactive chemicals are detected by the peripheral sensory neurons by activating TRPV1, TRPA1, or ASIC ion channels (TIH receptors). The activation of these channels induces neurogenic inflammatory and brain-mediated responses of the airways. These responses compromise breathing and can lead to disease states if they persist. The TIH receptors and the sensory neurons may be hypersensitized, leading to disorders of hypersensitive or chronic airway inflammation and cough. The channels may have a role in the pulmonary edema and other aspects of ARDS seen with high-level exposures to reactive TIHs. Therefore, targeting these channels may provide novel treatments to alleviate TIH-induced irritation, airway inflammation, disorders of chronic airway inflammation, cough, and perhaps even the ARDS seen with high-level exposure.

Conflict of Interest Statement: B.F.B. has a patent through Yale University on a method for preventing or alleviating the noxious effects resulting from toxicant exposure. S.-E.J. served as a consultant for Hydra Biosciences, Cambridge, MA (\$5,001–\$10,000) and served on their Advisory Board (\$1,001–\$5,000). He receives royalties from the University of California, San Francisco, for the Trpa1-/- mouse strain (\$1,001–\$5,000) and from Hydra Biosciences, Cambridge, MA for license negotiations.

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