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Altered ion transport in normal human bronchial epithelial cells following exposure to chemically distinct metal welding fume particles

Jeffrey S. Fedan *, Janet A. Thompson, Terence G. Meighan, Patti C. Zeidler-Erdely, James M. Antonini

Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA

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ABSTRACT

Welding fume inhalation causes pulmonary toxicity, including susceptibility to infection. We hypothesized that airway epithelial ion transport is a target of fume toxicity, and investigated the effects of fume particulates from manual metal arc-stainless steel (MMA-SS) and gas metal arc-mild steel (GMA-MS) on ion transport in normal human bronchial epithelium (NHBE) cultured in air-interface. MMA-SS particles, more soluble than GMA-MS particles, contain Cr, Ni, Fe and Mn; GMA-MS particles contain Fe and Mn. MMA-SS or GMA-MS particles (0.0167–166.7 μg/cm^2) were applied apically to NHBEs. After 18 h transepithelial potential difference (V_t), resistance (R_t), and short circuit current (I_{sc}) were measured. Particle effects on Na^+ and Cl^- channels and the Na^+,K^+,2Cl^--cotransporter were evaluated using amiloride (apical), 5-nitro-2-[(3-phenylpropyl)amino]benzoic acid (NPPB, apical), and bumetanide (basolateral), respectively. MMA-SS (0.0167–16.7 μg/cm^2) increased basal V_t. Only 16.7 μg/cm^2 GMA-MS increased basal V_t significantly. MMA-SS or GMA-MS exposure potentiated I_{sc} responses (decreases) to amiloride and bumetanide, while not affecting those to NPPB, GMA-MS to a lesser degree than MMA-SS. Variable effects on R_t were observed in response to amiloride, and bumetanide. Generally, MMA-SS was more potent in altering responses to amiloride and bumetanide than GMA-MS. Hyperpolarization occurred in the absence of LDH release, but decreases in V_t, R_t, and I_{sc} at higher fume particulate doses accompanied LDH release, to a greater extent for MMA-SS. Thus, Na^+ transport and Na^+,K^+,2Cl^--cotransport are affected by fume exposure; MMA-MS is more potent than GMA-MS. Enhanced Na^+ absorption and decreased airway surface liquid could compromise defenses against infection.

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1. Introduction

Inhalation of welding fume is associated with numerous morbidities in the pulmonary system of workers (Sferlazza and Beckett, 1991; Antonini et al., 2003, 2010). Several types of welding processes are utilized in manufacturing, and they give rise to fumes of different metal compositions and flux-derived materials (Antonini et al., 1999). In shielded manual metal arc-stainless steel (MMA-SS) welding, fluxes are incorporated into welding rods to protect the welds from oxidation. In gas metal arc-mild steel (GMA-MS) welding, shielding gases are used to protect against oxidation. The composition of the particles generated during MMA-SS and GMA-MS processes are different: MMA-SS particles contain Cr, Ni, Fe and Mn (28% Cr, 3% Ni, 41% Fe and 17% Mn, respectively), whereas GMA-MS particles contain Fe and Mn (85% and 14%, respectively) (Antonini et al., 1999, 2003). Antonini et al. (1999) also determined that MMA-SS fume particles are more soluble than GMA-MS particles (soluble/insoluble ratio of 0.345 for MMA-SS compared to 0.02 for GMA-MS).

A number of investigations have described differences in the toxicities of welding fumes of different composition in myriad organ systems. In a comparison of the relative toxicities of MMA-SS and GMA-MS fumes following intratracheal instillation of generated particles, Antonini et al. (2010) observed that MMA-SS evoked substantially more lung toxicity than GMA-MS, in terms of the cellular inflammatory response, and elevations in the levels in lung lavage of lactate dehydrogenase (LDH) activity and albumin; GMA-MS elicited only mild responses. These differences could have arisen from the greater water solubility of MMA-SS metals compared to GMA-MS. In a similar, comparative study addressing differences between manual metal arc-hard surfacing (MMA-HS) and GMA-MS, a greater toxicity of MMA-HS was observed in the heart, in which myocardial contractility and reactivity to the positive inotropic, β-adrenoceptor agonist, isoproterenol, were diminished to a greater degree by MMA-SS (Zheng et al., 2015). Consisting of Mn (50.9%) and Cr (8.5%), MMA-HS fume also has greater solubility than GMA-MS. Zeidler-Erdely et al. (2008) found in a comparison of two mouse strains a possible tumorigenic effect caused by gas metal arc-stainless steel (GMA-SS) fume that was not evident in

* Corresponding author at: Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA.
E-mail address: jsf2@cdc.gov (J.S. Fedan).

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animals treated with MMA-SS and GMA-MS fumes; GMA-SS has greater bio-persistence than the other two fumes. Lastly, in a comparison of the effects of MMA-SS, GMA-SS and GMA-MS, pulmonary inflammatory gene induction was greatest after treatment with MMA-SS, whereas the largest increase in expression of stress genes in aorta and heart occurred in response to MMA-SS, which was attributable, as least in part, to soluble Cr (Erdely et al., 2011).

Welders experience greater susceptibility to respiratory infections and an increase in mortality due to lobar pneumonia (Coggon et al., 1994; Antonini et al., 2004; Coggon and Palmer, 2016; Marongiu et al., 2016; Suri et al., 2016). A number of proposals have been advanced to explain this finding, including a recent one from a report by Suri et al. (2016) in which it was observed that adherence of pneumococcal bacteria and infection is increased in cultured cells and in vivo in mice. Differences have been observed in the effects of MMA-SS and GMA-MS on the clearance of bacteria from the lungs. Compared to GMA-MS, the clearance of *L. monocytogenes* after infection was reduced substantially following treatment of rats with fumes generated from MMA-SS, in association with inflammation and immune suppression in the lung (Antonini et al., 2004). MMA-SS and GMA-MS fumes also differed in their detrimental effects on macrophage function and viability: MMA-SS produced greater toxicity than GMA-SS (Antonini et al., 1999).

We hypothesized that clearance of bacteria from the airways by the mucociliary escalator may be retarded by exposure to welding fumes, which is an alteration that would prolong bacterial residence and foster greater adherence to airway cells. This could arise if the fume exerts a toxic effect on airway epithelium, so as to interfere with ion transport and the establishment of an airway surface liquid (ASL) that has a depth that is sufficient to allow adequate beating of cilia and clearance of bacteria from the lungs by the escalator. Such a dehydration of the ASL is thought to account for the chronic bacterial infection seen in cystic fibrosis patients (Haq et al., 2016). It is possible that some differences in lung toxicity caused by MMA-SS and GMA-MS fumes could reflect differences in the metal compositions and solubilities of the fumes. Thus, the present study was conducted to investigate the effects of MMA-SS and GMA-MS fume particles on ion transport in human primary cultured airway epithelial cells. Fume particle effects on concomitant LDH release also was investigated to ascertain effects on ion transport and cell damage occur concomitantly with respect to fume dose. The results indicate that welding fume particles interfere with ion transport in such a way as to favor increased transepithelial Na^+ absorption through ENaC. Associated with dehydration of the airway surface liquid, this effect could hinder efficient clearance of bacteria from the airways.

2. Materials and methods

2.1. Culture of normal human bronchial epithelial (NHBE) cells

NHBE cells (Lonza Inc.; Walkersville, MA) cells were seeded in plastic T-75 flasks and were grown in submersion in B-ALI medium (Lonza) until 80% confluent. The confluent monolayer was trypsinized for 4–6 min, and cells were seeded onto permeable inserts (0.4 μm pores; Corning; Corning, NY) that had been coated with rat tail collagen (BD Biosciences; San Jose, CA); cell density was 50,000 cells per insert. Cells were maintained at 37 °C in an air/5% CO_2 mixture in an incubator. NHBE cells were submerged for three days in B-ALI growth medium (100 μl apical chamber; 500 μl basal chamber) before 500 μl of B-ALI differentiation medium was added to the basal chamber and the apical medium removed to initiate the air-liquid interface (ALI) culture conditions. Medium was changed every 48 h. During cell growth and differentiation transepithelial resistance (R_t) was measured with EVOM^2 epithelial volt-ohm meter STX^2 electrodes (World Precision Instruments; Sarasota, FL) to assess growth to confluence from the increase in the R_t. Cells were ready for use at 24 d, when R_t reached a value of at least 1000 Ω cm^2.

2.2. Exposure of NHBE cells to welding fumes

The MMA-SS and GMA-MS fume particles used in this study were provided by Lincoln Electric Co. (Cleveland, OH). Details regarding the generation and characterization of these particles have been published (Zeidler-Erdely et al., 2008; Antonini et al., 1999). Count median diameters of the particles were 0.92 and 1.38 μm for MMA-SS and GMA-MS respectively. Particles that had been collected on filters were suspended in B-ALI differentiation medium and applied (50 μl) to the apical surface of the cells in doses ranging from 0.0167 to 166.7 μg/cm^2. Control cells received only differentiation medium. The NHBE cells were incubated under these conditions for 18 h before measurements were made. The lowest dose was equivalent to ~17 mg in a human lung deposition, calculated as ~2 days of human exposure at the previous threshold limit value-time-weighted average of 5 mg/m^3.

2.3. NHBE cell electrophysiology

After incubation with welding fumes, inserts containing adherent NHBE cells were placed into Ussing chambers in order to measure transepithelial potential difference (V_t; mV), using Ag/AgCl electrodes (0.9% NaCl in 4% agar). The apical and basolateral chambers contained modified Krebs-Henseleit (MKH) solution (composition below). The cells were allowed to reach a stable V_t under open-circuit conditions before applying a 0 mV voltage-clamp in order to record short-circuit current (I_{sc}; μA/cm^2) using an EVC 4000 automatic voltage/current amplifier (World Precision Instruments; Sarasota, FL). Square-wave voltage pulses (1 mV, 5 s duration) were delivered every 55 s to yield a voltage response for calculation of R_t from Ohm's law.

2.4. NHBE cell ion channel/transport blockers

Under short-circuit conditions, responses (I_{sc} and R_t) of the cells were measured after administration of the epithelial Na^+ channel blocker, amiloride (3.5×10^{-5} M), applied to the apical chamber of the Ussing chamber; the Cl^- channel blocker, 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB, 10^{-4} M), applied to the apical chamber; and bumetanide (10^{-4} M), the Na^+,K^+,2Cl^--cotransport blocker, applied to the basolateral chamber. These agents evoked electrophysiological responses, which were quantified as percent change from I_{sc} values before their addition, i.e., %Δ I_{sc} and %Δ R_t.

2.5. Measurement of LDH activity

The oxidation of lactate to pyruvate and the formation of NADH was measured in samples from the apical and basolateral compartments of the transwell inserts. Medium was harvested from four inserts for each dose of fume particles or controls. 100 μl of B-ALI differentiation medium was first added to the apical compartment, which had been given 50 μl with the fume treatment, from which 150 μl was retrieved for assay of LDH activity. LDH measurements were made using a COBAS c111 analyzer (Roche Diagnostic Systems; Indianapolis, IN).

2.6. Chemicals and reagents

MKH solution (pH 7.4, 37 °C) contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 5.7 mM glucose, and was saturated with 95% O_2/5% CO_2. Amiloride, NPPB, and bumetanide were dissolved in saline. All drugs and reagents were from Sigma-Aldrich (St. Louis, MO).

2.7. Statistical analysis

The results are expressed as means ± S.E.M of replicate determinations using separate inserts for Ussing chamber and LDH measurements.

n values are given in the results. The results were analyzed for differences using ANOVA. $^*P < 0.05$ was regarded as significant.

3. Results

3.1. Effects of welding fumes on basal bioelectric characteristics of NHBE cells

The purpose of these experiments was to investigate whether incubation of NHBE cells with MMA-SS or GMA-MS would evoke bioelectric changes in NHBE, and to compare the two fumes for their ability to cause this effect. The results obtained using Ussing chambers are shown in Fig. 1. Both fumes caused two types of V_t, responses, i.e., hyperpolarization and depolarization (Fig. 1, top panel); hyperpolarization occurred at lower fume doses than those causing depolarization. Responses to MMA-SS differed from those to GMA-MS in two respects: MMA-SS caused hyperpolarization at the three lowest doses (0.0167–1.67 μg/ml), whereas a hyperpolarizing effect was not observed with GMA-MS except at 16.7 μg/ml (a seeming effect at 1.67 μg/ml was not significant). Second, both 16.7 and 167 μg/ml MMA-SS decreased V_t, and, in fact, V_t was abolished by 167 μg/ml MMA-SS but not reduced by 167 μg/ml GMA-MS. Thus, MMA-SS was more potent than GMA-MS both in increasing and decreasing V_t.

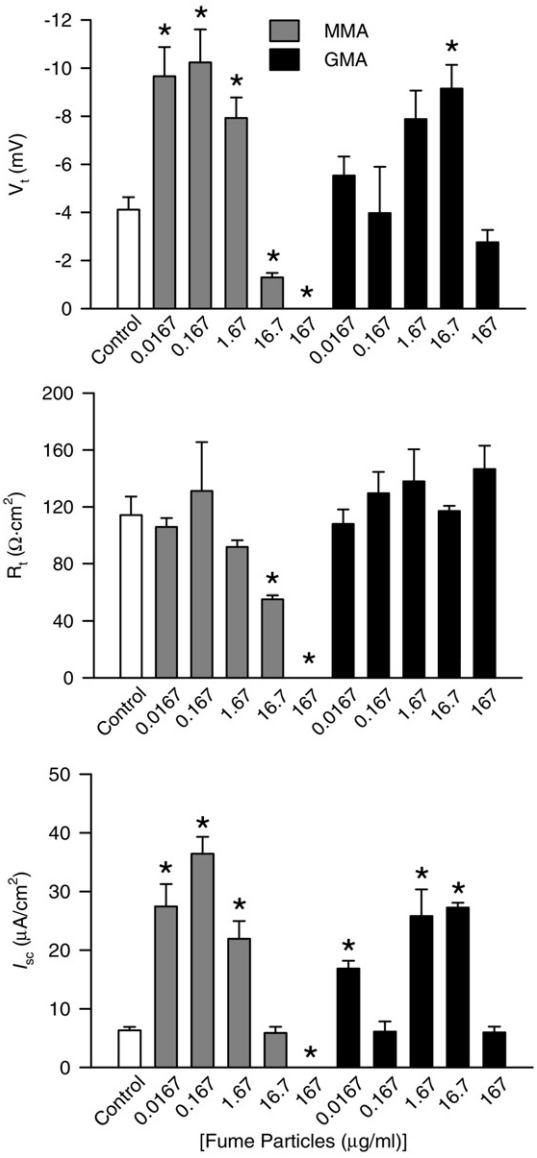

Fig. 1. Effects of MMA-SS (MMA) and GMA-MS (GMA) on V_t (top panel), R_t (middle panel) and I_{sc} (bottom panel) following an 18-h incubation with cultured NHBE cells. No V_t or R_t or, hence, I_{sc}, were measurable after incubation with 167 μg/ml MMA-SS. $^*P < 0.05$ compared to Control (B-ALI differentiation medium). Control, $n = 16$. *n* values for MMA-SS experiments were 16–20. *n* values for GMA-MS experiments were 8–12.

The increases and decreases in V_t could have reflected alterations in active ion transport and/or passive transport through the paracellular pathway of the epithelium resulting from perturbations in tight junctions, which would affect transepithelial resistance. Therefore, the effect of fume exposure on R_t was investigated, with an emphasis on comparing MMA-SS and GMA-MS. Fig. 1 (middle panel) indicates that MMA-SS decreased R_t at 16.7 and 167 μg/ml, whereas GMA-MS had no effect on R_t. These findings suggest, firstly, that the hyperpolarization induced by both types of fumes did not result from changes in R_t, and, secondly, the depolarization of NHBE cells by 16.7 and 167 μg/ml MMA-SS appeared to be attributable to declining tight junction integrity and R_t.

Examination of the effects of MMA-SS and GMA-MS on I_{sc} gave insight into the effects of the fumes on active ion transport, which was suggested by their effects on V_t. Fig. 1 (bottom panel) illustrates that the fume concentrations (0.0167–1.67 μg/ml) that had hyperpolarized the epithelium (Fig. 1, top panel) also increased I_{sc}. Current was reduced to the control level at 16.7 μg/ml MMA-SS, in keeping with the decreased V_t observed at this concentration (Fig. 1, top panel), despite the reduction in R_t that had occurred (Fig. 1, middle panel). In the case of GMA-MS, I_{sc} was increased at all but 0.167 (which is unexplained) and 167 μg/ml (Fig. 1, bottom panel), at which I_{sc} was abrogated; occurring in the absence of a fall in R_t, (Fig. 1, middle panel), active ion transport appears to have been inhibited at this fume concentration. Thus, the effects of MMA-SS and GMA-MS on I_{sc} essentially mirrored their effects on V_t, but MMA-SS had greater inhibitory effects at higher concentrations.

3.2. Effects of ion transport inhibitors

The above findings suggested that epithelial active ion transport is affected by incubation with welding fume particles. To investigate the ion channels and transporters that were influenced by fumes, a series of experiments was conducted to evaluate the effects of fume particle exposure on I_{sc} responses to transport inhibitors. In control NHBE cells incubated with the vehicle (B-ALI differentiation medium) in which welding fume particles were suspended, the ENaC inhibitor, amiloride (3.5×10^{-5} M) applied to the apical bath caused an approximately 40% decrease in I_{sc} (Fig. 2, top panel); R_t was unaffected (Fig. 2, bottom panel). Added to cells that had been incubated with welding fume particles, amiloride produced an inhibition of I_{sc} that was twice as large as control (Fig. 2, top panel), suggestive of a heightened transport of Na^+ through ENaC. This effect was somewhat larger for MMA-SS compared to GMA-MS. Interestingly, R_t, which had not been affected in the control cells, was significantly increased by amiloride after incubation with both MMA-SS and GMA-MS, the magnitude of this effect being greater at low concentrations of MMA-SS compared to GMA-MS (Fig. 2, bottom panel). At 16.7 μg/ml MMA-SS the stimulatory effects on I_{sc} and R_t were not evident. It is of note that I_{sc} was increased by both fumes in the face of concomitant elevations in R_t. [No data are shown for 167 μg/ml MMA-SS, as there was no measurable V_t or R_t at this concentration of fume (Fig. 1, top panel and middle panels, respectively)].

A similar experiment was performed with the Cl^- channel blocker, NPPB (10^{-4} M), applied to the apical chamber. In control cells, NPPB reduced I_{sc} by ~80%, indicating that in NHBE cells Cl^- is the major ion contributing to basal V_t. In cells incubated with MMA-SS, no effect of NPPB on I_{sc} was observed (Fig. 3, top panel). In contrast, exposure to the higher doses of GMA-MS inhibited the NPPB-induced decrease in I_{sc}, and this effect progressed with increasing GMA-MS concentration, being significant at 167 μg/ml GMA-MS. NPPB appeared to increase slightly R_t at low concentrations of MMA-SS and GMA-MS, and had no effect at the higher fume particle levels (Fig. 3, bottom panel). However,

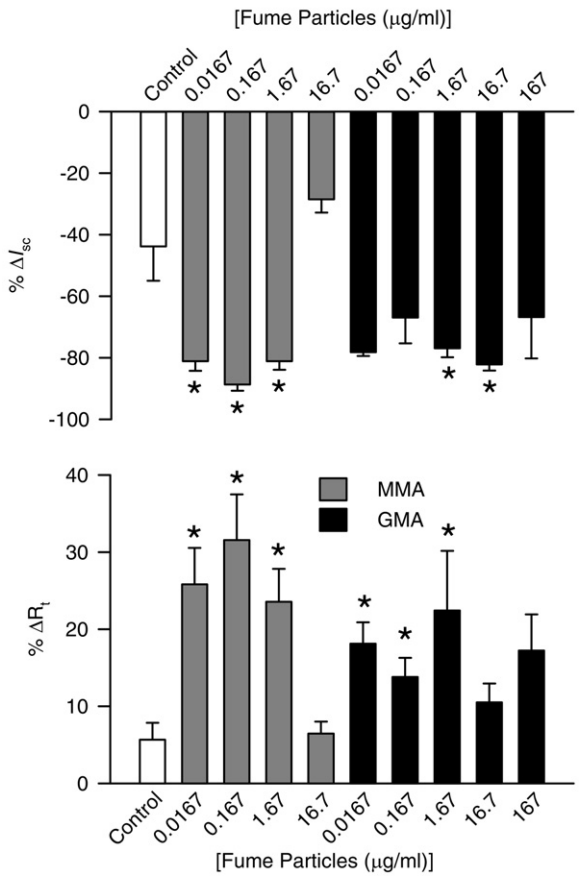

Fig. 2. Effects of amiloride (3.5×10^{-5} M; apical) on I_{sc} (top panel) and R_t responses (bottom panel) following an 18-h incubation of cultured NHBE cells with MMA-SS (MMA) or GMA-MS (GMA). The I_{sc} and R_t values are expressed as percent change from their values before the addition of amiloride, i.e., %Δ I_{sc} and %Δ R_t. Amiloride decreased I_{sc} in control cells incubated with B-ALI differentiation medium, reflective of inhibition of Na^+ transport through ENaC. $^*P < 0.05$ compared to control. Control, $n = 6$. n values for MMA-SS experiments were 6–8 in each condition. n values for GMA-MS experiments were 4–7.

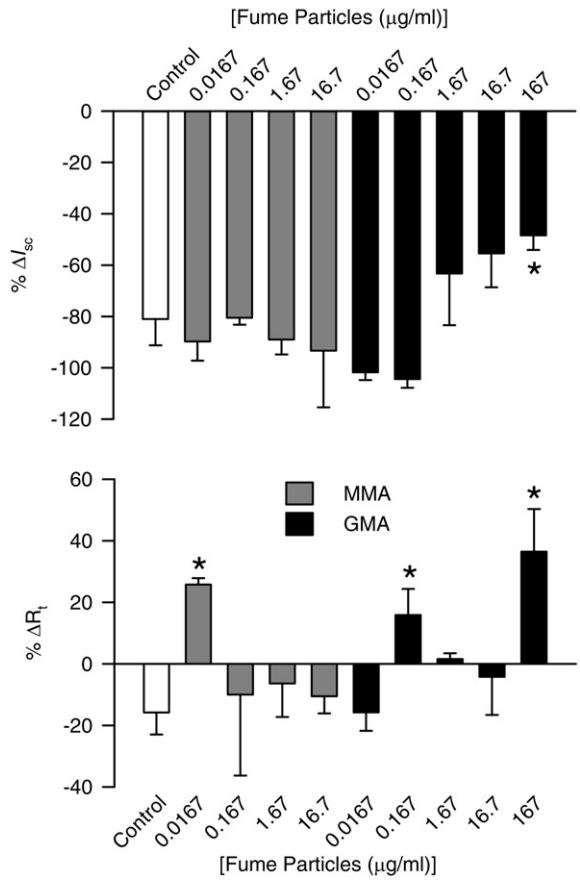

Fig. 3. Effects of NPPB (10^{-4} M; apical) on I_{sc} (top panel) and R_t responses (bottom panel) following an 18-h incubation of cultured NHBE cells with MMA-SS (MMA) and GMA-MS (GMA). The I_{sc} and R_t values are expressed as percent change from their values before the addition of NPPB, i.e., %Δ I_{sc} and %Δ R_t. NPPB decreased I_{sc} in control cells incubated with B-ALI differentiation medium, reflective of inhibition of Cl^- transport through Cl^- channels. $^*P < 0.05$ compared to control. Control, $n = 6$. n values for MMA-SS experiments were 6–8 in each condition. n values for GMA-MS experiments were 4–8.

an increase in R_t at 167 μg/ml GMA-MS coincided with, and could explain, the observed decrease in I_{sc} at this dose (Fig. 3, top panel).

The effect of basolaterally-applied bumetanide (10^{-4} M), an inhibitor of Na^+,K^+,2Cl^--cotransport, on NHBE cells is shown in Fig. 4. Bumetanide halved I_{sc} (Fig. 4, top panel). In cells which had been incubated with MMA-SS and GMA-MS, the fume particles generally augmented the effect of bumetanide; however, in cells incubated with 16.7 μg/ml MMA-SS, the I_{sc} response was attenuated significantly. In a manner similar to that observed with amiloride (Fig. 2, bottom panel), bumetanide increased R_t in cells incubated with 0.0167–1.67 μg/ml MMA-SS, but not with 16.7 μg/ml MMA-SS. In the case of GMA-MS, R_t was increased at 0.0167 and 0.167 μg/ml, but it was of interest that, while I_{sc} was decreased at 1.67 and 16.7 μg/ml GMA-MS, R_t was not significantly affected.

Overall, the results of the bioelectric studies would indicate that Na^+ transport through ENaC and Na^+,K^+,2Cl^--cotransport were affected by MMA-SS and GMA-MS, and suggest that the effects of welding fume particles on basal epithelial ion transport (Fig. 1) involved alterations in these transport pathways.

3.3. Cytotoxicity of MMA-SS and GMA-SS: bioelectric changes vs. LDH release

Incubation of alveolar macrophages with MMA-SS and GMA-MS neat fume particles, and their soluble and insoluble fractions, has been reported to elicit LDH release (Antonini et al., 1999), indicative of cytotoxicity to these cells. Therefore, whether MMA-SS and/or GMA-MS particles cause LDH release in NHBE cells was investigated. In particular, it was of interest to evaluate whether any relationship could be ascribed between the effects of the particles on ion transport and on cytotoxicity. In these experiments samples were taken from the apical and basolateral compartments of the transwell inserts for measurement of LDH release. The results are shown in Fig. 5. For both types of particles, LDH release was polarized and occurred primarily into the apical compartment, the release into the basolateral compartment being below the limit of detection. In response to incubation with MMA-SS the NHBE cells did not release LDH into the media at concentrations ≤ 1.67 μg/ml. This dose range corresponded to that over which V_t and I_{sc} were increased by MMA-SS (Fig. 1). GMA-MS did not cause LDH release, and, in fact, reduced release at 167 μg/ml. Thus, there was no LDH release at concentrations at which V_t and I_{sc} were heightened by the particles (Fig. 1). Thus, for both types of fume, the activation of ion transport by MMA-SS and GMA-MS is not associated with LDH release and, therefore, cytotoxicity, whereas inhibition of ion transport by the fumes occurs in association with cytotoxicity.

4. Discussion

In this investigation evidence was obtained to indicate that 1) both MMA-SS and GMA-MS in lower concentrations stimulate active ion transport in human airway epithelial cells without altering transepithelial resistance, 2) MMA-SS appears to be more potent than

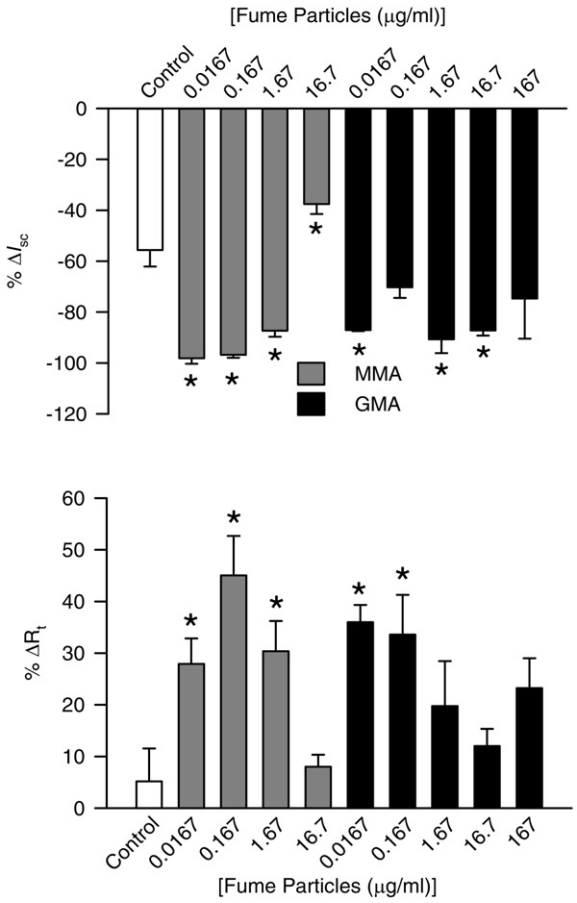

Fig. 4. Effects of bumetanide (10^{-4} M basolateral) on I_{sc} (top panel) and R_t responses (bottom panel) following an 18-h incubation of cultured NHBE cells with MMA-SS (MMA) and GMA-MS (GMA). The I_{sc} and R_t values are expressed as percent change from their values before the addition of bumetanide, i.e., %Δ I_{sc} and %Δ R_t. Bumetanide decreased I_{sc} in control cells incubated with B-ALI differentiation medium, reflective of inhibition of the Na^+,K^+,2Cl^--cotransporter. *$P < 0.05$ compared to control. Control, $n = 6$. n values for MMA-SS experiments were 6–8 for each condition. n values for GMA-MS experiments were 4–7.

GMA-MS with regard to activation of ion transport, 3) elevations of V_t and I_{sc} initiated by both MMA-SS and GMA-MS exposure occur primarily as a result of an increase in amiloride-sensitive Na^+ current through ENaC and also involves the bumetanide-sensitive Na^+,K^+-cotransporter, but not Cl^- channels, 4) higher concentrations of MMA-SS reduce markedly R_t, whereas GMA-MS is devoid of this effect, 5) stimulation of active ion transport by the fumes occurs in the absence of an effect on cell viability, as judged from the absence of LDH release under these conditions, 6) higher concentrations of MMA-SS induces cytotoxicity, in terms of LDH release as a marker, whereas GMA-MS is less active in this regard and, 7) in general, MMA-SS is more toxic to airway epithelial cells than GMA-MS.

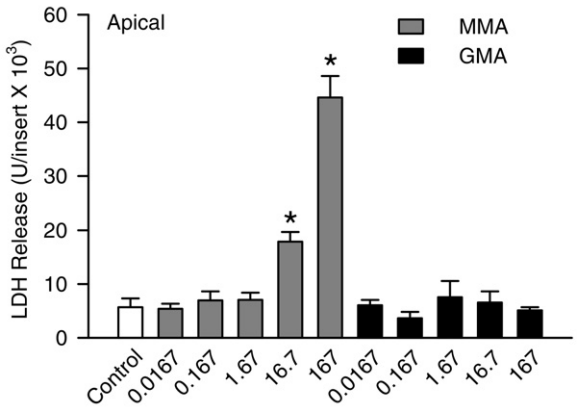

Fig. 5. Effect of incubation in MMS-SS (MMA) or GMA-MS (GMA) on LDH release into the apical (top panel) or basolateral (bottom panel) chambers of cultured NHBE cells. LDH release into the basolateral chamber was below the limit of detection. *$P < 0.05$ compared to control.

The greater potency of MMA-SS in activating ion transport may reside in the heightened solubility of these fume particles compared to GMA-MS and/or the fact that they contain two metals, Cr, most of which is soluble, and Ni, which are not present in GMA-MS. Nevertheless, GMA-MS did have similar efficacy as MMA-SS, albeit at higher concentrations. Thus, the presence of soluble Cr and/or Ni cannot per se be the sole determinant of whether ion transport is stimulated by fume particles, but potency was increased by the presence of these metals. This finding supports those of Erdely et al. (2011), who also considered insoluble components of welding fume to contribute to their cytotoxic effects.

A strong relationship exists between the presence of soluble Cr and Ni in fume particles and the observed decline in transepithelial resistance, inasmuch as MMA-SS caused a dose-related decline in R_t, whereas R_t was not affected at any dose of GMA-MS. As well, the decreases in R_t followed the same dose-relationship as the release of LDH from the cells. However, at several MMA-SS doses (0.0167–1.67 μg/ml) V_t and I_{sc} were elevated although LDH release did not occur. This suggests that there is little, if any, direct relationship between stimulation of ion transport by the fume particles and their cytotoxicity, assessed as LDH release. It would appear that separate mechanisms account for ion transport activation and cytotoxicity.

An important finding of this investigation was the stronger inhibition of I_{sc} by amiloride following treatment with both types of fume particles than was observed in the unexposed NHBE cells. This finding suggests that Na^+ transport through ENaC was potentiated by fume particle exposure. At the same time, Cl^- transport appeared not to be influenced by the particles, as judged from the lack of effect of NPPB on I_{sc}. There is no reason to suspect that sufficient fume particles were present in the modified Krebs-Henseleit solution bathing the cells that could have solubilized to a degree to complex with NPPB. Assuming that some particles did remain on epithelial cells in the Ussing chamber, the molar ratio of NPPB to metals would be very large considering the trace amount of the soluble metals that might be present. As well, binding of soluble metals derived from fume particles to NPPB has not been reported. The inhibitory effect of bumetanide on I_{sc} also was heightened after fume particle exposures, possibly signifying that basolateral exit of Na^+ from the NHBE cells via basolateral membrane Na^+,K^+-2Cl^- cotransport was facilitated to accommodate enhanced apical Na^+ entry through ENaC.

Granted that both types of welding fumes increased V_t and I_{sc} by enhancing Na^+ entry through ENaC, whether MMA-SS is more potent in this regard than GMA-MS because the particles contain Cr and Ni is worth consideration. As well, a more general question is to ask whether it can be established whether Fe or Mn is involved in stimulation of Na^+ transport. Inasmuch as both types of fumes stimulated Na^+ transport, and both contain Fe and Mn, either of these metals could be considered as causative. Unfortunately, we were unable to identify reports in the literature of interactions of Fe or Mn with ENaC. The greater potency of MMA-SS compared to GMA-MS suggests that the presence of Cr and Ni could interact with ENaC to potentiate the stimulation initiated by Fe and Mn. Whereas there are no reports in the literature regarding the interaction of Cr with ENaC, several investigations have found that Ni^{2+} interacts with and alters performance of ENaC. In two studies (Sheng et al., 2002, 2004) in which cDNAs of *mouse* ENaC subunits were inserted into *Xenopus* oocytes, Ni^{2+} was observed to inhibit ENaC by decreasing channel open probability and also relieving ENaC from self-inhibition by Na^+. The significance of these findings is that they would predict that Ni^{2+} would reduce, rather than increase, V_t

and I_{sc}, which was the finding in the present study. In a comparison of the effects of Ni^{2+} on ENaC from *Xenopus* or rat colon expressed in oocytes (Cucu et al., 2005), Ni^{2+} stimulated Na^+ permeability through the amphibian ENaC, but inhibited the channel derived from rat, as had been observed earlier in mouse ENaC (Sheng et al., 2004). It is, therefore, difficult to reconcile the published effects of Ni^{2+} with the enhanced effectiveness of amiloride on activate ion transport observed in the present study. It remains possible that Cr may stimulate Na^+ transport through ENaC, and this notion will require further investigation. It is also worth considering the possibility that the physical contact of fume particles with the epithelial cell triggers Na^+ entry through unknown transduction pathways.

While the mechanism whereby fume particles stimulate amiloride-sensitive Na^+ transport remains unclear, the deleterious effects of MMA-SS but not GMA-MS on R_t and cell integrity appears to be attributable to the presence of soluble Cr and Ni in the fume. The damage is such that the epithelium cannot maintain vectorial ion transport. As well, the dose-related release of LDH signifies that damage to the epithelial cells is appreciable.

The polarized release of LDH into the apical medium following exposure to MMA-SS is of interest. There are two possible explanations for this difference. In the first, which we favor, damage to the epithelium occurred predominantly to the apical membrane of the cells because the fume particles were resident there, with little communication of the contact event to the basolateral membrane. Some soluble Cr and Ni could permeate the cells and perturb the basolateral membrane to cause LDH release. If the latter occurs, it is not an especially strong effect because the concentrations of the metals at the basolateral membrane would be attenuated. In the second scenario, the amount of LDH diffusing into the basolateral medium could be less simply because the surface area of the basolateral membrane in contact with the 0.4 μm pores in the insert membrane is a very small percentage of the entire basolateral membrane surface, and diffusion of LDH into the basolateral medium would be constrained. In contrast, release of LDH into the apical compartment is relatively unhindered. In preliminary experiments using cells from a different patient, a small amount of LDH was detected in the basolateral medium (4–10 units/insert).

Finally, the primary result of this investigation, mainly, that MMA-SS and GMA-MS activate Na^+ transport, offers some insight into why welding workers are more susceptible to infection than the general population. We suggest that enhanced Na^+ entry into epithelial cells results in a dehydration of the airway surface liquid, the sequelae of which includes reduction in the height of the airway surface liquid and thickening of mucus, which would reduce the effectiveness of cilia to carry infectious microbes up the muco-ciliary escalator. Differences in the pharmacological characteristics of MMS-SS and GMA-MS were also observed with regard to their potency as activators of Na^+ transport and with regard to changes in transepithelial ion transport and LDH release. Thus, this investigation has contributed additional information about these fumes that complements earlier reports of differences noted in the lung and cardiovascular system.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health. Mention of brand name does not constitute product endorsement.

Conflict of interest statement

The authors declare that they have no conflicts of interest in relation to this publication.

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