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# Hard Metal-Induced Disease: Effects of Metal Cations *in Vitro* on Guinea Pig Isolated Airways[1]

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**Inhalation of dust from hard metal (HM), a mixture of tungsten carbide, cobalt, and other metals, can cause interstitial alveolitis, fibrosis, and asthma in the workplace. Some effects of HM could occur after the metals dissolve in the lung. We examined whether chloride salts of metals in HM alloys can elicit responses or modify reactivity to methacholine (MCh) or responses to electric field stimulation (EFS) in guinea pig tracheal strips. In unstimulated strips, $Co^{2+}$, $Cd^{2+}$, and $Ni^{2+}$ evoked contractions ($>3 \times 10^{-6}$ M), while $Ta^{5+}$, $Zn^{2+}$, $Cr^{2+}$, and $Cr^{3+}$ caused weak relaxations ($>10^{-5}$ M). In strips contracted with MCh ($3 \times 10^{-7}$ M), $Co^{2+}$ and $Ni^{2+}$ also caused relaxation in lower concentrations while the other metals caused weak relaxation only in high concentrations ($>10^{-4}$ M). The metals were generally without effect on reactivity to MCh, except that $Cd^{2+}$ inhibited and $Ni^{2+}$ potentiated some responses. The effects of selected metals ($10^{-6}$ M; $Cr^{3+}$, $Ni^{2+}$, $Cd^{2+}$, and $Co^{2+}$) on EFS-induced contractile and relaxant responses were examined (±MCh; $\pm 10^{-6}$ M indomethacin (Indo), 30 min). No metal had any effect on the excitatory nonadrenergic, noncholinergic-mediated contraction phase. $Cd^{2+}$ and $Ni^{2+}$ inhibited cholinergically mediated contractions of unstimulated strips (+Indo), whereas $Cr^{3+}$ both inhibited (−MCh, −Indo) and potentiated (−Indo,+MCh; +Indo, +MCh) contractile responses. $Cr^{3+}$ was the only metal to inhibit the inhibitory nonadrenergic, noncholinergic-mediated relaxation phase (±MCh; −Indo). $Co^{2+}$ had no effect at all. The results suggest that smooth muscle tone and nerves in the airways could be targets of cationic metals after they dissolve in the lung.**

*Key Words:* **hard metal; guinea pig; trachea; methacholine; electric field stimulation; cholinergic nerves; nonadrenergic, noncholinergic nerves; airway reactivity; tantalum; cobalt; zinc; cadmium; chromium; nickel.**

Inhalation of hard metal (HM) may cause interstitial lung disease (hard metal disease) (Sprince *et al.,* 1988; Lison, 1996), lung irritation (Kusaka *et al.,* 1986), fibrosis, and occupational asthma (Bernstein *et al.,* 1999) in the workplace. HM has a hardness near that of diamond and is used widely in manufacturing. HM is a mixture of primarily tungsten carbide (WC) and cobalt metal, as well as other metals such as Zn, Cd, Cr, Co, Ta, Ni, and others, in lesser amounts, as specified by metallurgic requirements.

The etiology of the diseases precipitated by inhaled HM is not known but has been investigated in animal models. In many respects the animal studies involving intratracheal instillation of HM particles or mixtures of WC and Co have recapitulated several, but not all of the hallmarks of the interstitial disease, edema, and inflammation observed in humans (Schepers, 1955a,b,c; Kerfoot *et al.,* 1975; Lison, 1996; Adamis *et al.,* 1997; Rengasamy *et al.,* 1999). It has been established that WC–Co mixtures are more toxic to the lung than WC or Co administered separately (Lasfargues *et al.,* 1992, 1995; Barceloux, 1999b). While it has not been investigated for all the metals present in small amounts in alloys, Co has relatively high solubility in the lungs (Cugell, 1992; Ruokonen *et al.,* 1996), and, after intratracheal instillation, it has been observed that coadministered WC accelerates the clearance of Co from the lung (Lison and Lauwerys, 1994). Studies in our laboratory showed that instillation of HM caused up-regulation of nitric oxide (NO) synthase in the lung (Rengasamy *et al.,* 1999). Metals found in lesser amounts than Co in HM, e.g., Ni, Cd, Zn, and Cr, are toxic to the lungs in their own right following inhalation or intratracheal administration (Barceloux, 1999a,c,d; Derelanko *et al.,* 1999; Bell *et al.,* 2000).

A few studies have been conducted to investigate the effects of WC and WC–Co in *in vitro* systems. Lison (1996) observed that WC–Co gave rise to greater release of lactate dehydrogenase, a marker for cell damage, than was observed when macrophages were treated with WC or Co separately. Tian and

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[3] Abbreviations used: EFS, electric field stimulation; eNANC, excitatory nonadrenergic, noncholinergic; HM, hard metal; iNANC, inhibitory nonadrenergic, noncholinergic; MCh, methacholine; MKH, modified Krebs—Henseleit.



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Lawrence (1996) examined $Cd^{2+}$, $Co^{2+}$, $Cr^{3+}$, $Ni^{2+}$, and $Zn^{2+}$ (chloride salts) for their ability to alter NO production by macrophages. They observed diversity in the effects of the metal cations, in that $Cd^{2+}$ inhibited NO production, while $Cr^{3+}$ and $Zn^{2+}$ were without effect, and $Ni^{2+}$ and $Co^{2+}$ increased NO production. In cell-free systems, HM (Rengasamy *et al.*, 1999) and Cr and Zn chlorides (Tian and Lawrence, 1996) were found to interfere with NO synthase activity. It has been suggested that the mechanism of toxicity of HM/WC–Co may involve the generation of free radicals (Lison *et al.*, 1995). Indeed, HM alloy constituents, such as Cd, may also give rise to free radicals, which may cause cellular damage (Hassoun and Stohs, 1996).

As indicated above, a major emphasis of previous studies of HM-induced diseases has been on the inflammatory response. We observed in addition to this response that instillation of HM resulted in hyporeactivity of treated animals to inhaled methacholine (Rengasamy *et al.*, 1999). The relatively rapid clearance of Co from the lung, when administered as WC–Co or Co alone, suggests that dissolution of the metal could give rise to local concentrations high enough to affect cells, i.e., smooth muscle cells and efferent nerves, that are involved in the control of airway diameter. There has been little work on the effects of alloy metals on airway smooth muscle function and reactivity; most published work has been done in the context of metals as probes to examine $Ca^{2+}$ channels, i.e., Ni (Amrani *et al.*, 1994; Cuthbert *et al.*, 1994; Yang *et al.*, 1994; Janssen, 1997; Madison *et al.*, 1998), Cd (Kotlikoff, 1988; Sarria *et al.*, 1989; Takemoto *et al.*, 1998), and Co (Kotlikoff, 1988). Vassilev *et al.* (1993) did observe that feeding $Co^{2+}$ or $Ni^{2+}$ salts led to a decrease in reactivity of rat tracheal strips. We uncovered no published studies of the effects of alloy metals, either *in vitro* or after treatment of animals, on neural control of the airways. With this knowledge in mind, the current study was designed to test the hypothesis that metals in HM alloys, once dissolved in the lung, can affect airway smooth muscle directly, the reactivity of the smooth muscle to bronchoconstrictor drugs, and the regulation of the smooth muscle by excitatory and inhibitory innervation. This hypothesis was tested *in vitro* using tracheal strips from guinea pigs to determine whether soluble salts of Ta, Co, Zn, Cd, Cr, and Ni can affect tone directly, alter reactivity to the contractile effects of methacholine (MCh), or influence contractile and relaxant responses mediated by cholinergic, parasympathetic postganglionic nerves and excitatory (eNANC) and inhibitory (iNANC) nonadrenergic, noncholinergic neurons.

## MATERIALS AND METHODS

***Animals.*** These studies were conducted in facilities accredited fully by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Male English short-hair SPF guinea pigs (400–600 g) were obtained from Harlan Sprague–Dawley, Inc. (Indianapolis, IN). The animals were anesthetized with sodium pentobarbital (65 mg/kg ip) and euthanized under anesthesia by thoracotomy and bleeding. A total of 108 animals were used in this study.

***Preparation of tracheal strips.*** A length of trachea was removed, placed in gassed modified Krebs–Henseleit (MKH solution; composition below) and cleaned. The trachea was slit through the cartilage along its longitudinal axis, and strips two cartilage rings in width were prepared. Particular care was taken not to damage the epithelium (Hay *et al.*, 1986). The strips were placed in water-jacketed organ baths (37°C) containing MKH solution and tied to force-displacement transducers for the measurement of isometric tension. The tissues were equilibrated for 1 h under an optimum resting load of 1*g*, and washed every 15 min before the start of the experiment. The modified MKH solution contained (in mM) NaCl 113, KCl 4.8, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, and glucose 5.5. The solution was gassed with 95% $O_2$–5% $CO_2$ to give a pH of 7.4 at 37°C.

***Concentration–response curves for cationic metals in resting and contracted preparations.*** Cumulative concentration–response curves were obtained for the chloride salts of the following metal cations: $Ta^{5+}$, $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Cr^{2+}$, $Cr^{3+}$, and $Ni^{2+}$. Curves were obtained from preparations under resting force and from preparations in which force was developed. In preparations examined while under basal force, a reference contraction was obtained at the beginning of the experiment by challenging the preparations with 120 mM KCl. After the plateau of the response to KCl was reached, the strips were washed with fresh MKH at 15-min intervals for 1 h. At this point the metal chlorides were added cumulatively to obtain the concentration–response relationship. In this protocol the responses to the metals were quantified and normalized as a percentage of the response to 120 mM KCl (% KCl in the figures). Inasmuch as strips of guinea pig trachea develop spontaneous tone (Hay *et al.*, 1986), the preparations were capable of relaxing below the baseline force (see figures). In other preparations in which tone was induced to examine the relaxant capability of the metals under more controlled conditions, the strips were contracted with the addition of $3 \times 10^{-7}$ M MCh, a concentration that approximates the EC50 (M) for contraction (Hay *et al.*, 1986). Responses to the metals in this protocol were quantified and normalized as a percentage of the force induced by MCh (% MCh). In both cases (i.e., MCh absent or present) the response to a given concentration of metal was allowed to reach a plateau before the next higher concentration was added. Concentration–response curves obtained from preparations at basal force as well as from preparations that had been contracted with MCh were obtained using different strips from the same trachea. The upper limit of metal concentrations that could be tested was limited by the formation of cloudiness or precipitates in the MKH solution as solubility products were exceeded; the concentrations at which these events occurred are denoted in the figures as C or P, respectively. These concentrations were determined in separate experiments (tracheal strips omitted from the organ chambers) in which the metals were added in cumulative concentrations to the organ chambers while watching for the development of cloudiness or a precipitate. These experiments were repeated six times for each of the seven metals, with separate tracheas used for each metal, and both conditions (i.e., ±MCh) were studied in each trachea.

***Effect of metal chlorides on MCh concentration–response curves.*** Strips obtained from the same animals were contracted with 120 mM KCl to obtain a reference contraction. After 1 h of washing with fresh MKH solution at 15-min intervals, strips were incubated for 1 h with either $10^{-6}$ M or $3 \times 10^{-4}$ M $Ta^{5+}$, $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Cr^{2+}$, $Cr^{3+}$, or $Ni^{2+}$ chlorides. The control strips received no treatment. At the conclusion of the incubation, MCh was added in cumulative concentrations to develop the concentration–response curve. The responses were quantified and normalized as a percentage of the KCl reference response (% KCl). These experiments were repeated six times for each of the seven metals, with separate tracheas used for each metal and the three conditions (control and two metal concentrations) were studied in each trachea.

***Effect of metal chlorides on frequency-response curves.*** The tracheal strips were first contracted with 120 mM KCl to establish a reference response. After washout of KCl (1 h), one group of tracheal strips from each animal was allowed to remain under resting force while another group of strips from the same animals was contracted with MCh ($3 \times 10^{-7}$ M) before applying

electrical field stimulation (EFS) to evoke endogenous neurotransmitter-mediated responses. The strips were stimulated at 7-min intervals with 10-s trains of pulses (130 V, 0.5 ms duration) of varying frequencies (0.3 to 30 Hz) to develop frequency–response curves. Frequency–response relationships for EFS-induced responses, in preparations under basal force or contracted with MCh, were obtained in the absence or presence of the cyclooxygenase inhibitor indomethacin ($3 \times 10^{-6}$ M). The indomethacin was added 30 min before the frequency–response determination of resting preparations or 30 min before the addition of MCh, as appropriate. Indomethacin relaxed the strips to passive force (Hay *et al.*, 1986). The effects of $Co^{2+}$, $Cr^{3+}$, $Cd^{2+}$, or $Ni^{2+}$ ($10^{-6}$ M) were examined after addition of the cations before indomethacin and/or MCh, after an incubation lasting a total of 1 h. The responses of preparations under basal force were quantified and normalized as a percentage of the reference response to KCl (% KCl); the responses of preparations that had been contracted with MCh were quantified and normalized as a percentage of the response to MCh (% MCh). Tracheas from six separate animals for each of the four metals were used in these experiments, with all conditions studied in each trachea.

The rationale for these experimental conditions was as follows. In tracheal strips, EFS evokes multiphasic responses consisting of a rapid, transient, cholinergically mediated contraction followed by a slower-developing relaxation below baseline in response to activation of iNANC terminals. The latter responses are mediated, in part, by nitric oxide and a neuropeptide, perhaps vasoactive intestinal polypeptide (Kesler and Canning, 1999; Moffat *et al.*, 1999). In some preparations, and depending on the stimulation frequency, a second more slowly developing contraction that is mediated by eNANC neurotransmitters, thought to be tachykinins, could be seen (Renzetti *et al.*, 1992). We were interested in examining the effects of the metals on all response phases. Because indomethacin caused relaxation of spontaneous tone, it was not possible to examine metal effects on EFS-induced relaxation in these preparations. Therefore, metal effects on EFS-induced relaxation in the presence of indomethacin were examined using MCh-contracted strips only.

***Analysis of data.*** The results are presented as $\bar{x} \pm$ SE. EC50 values for MCh were obtained using a four-parameter logistic curve fit (SigmaPlot). The effects of metals in two different concentrations on the MCh EC50 were compared using log-normally distributed $-\log$EC50 (M) values and one-way repeated-measures analysis of variance (ANOVA-RM). The effects of two concentrations of metals on responses to MCh were also evaluated using ANOVA-RM. The effects of metals on responses to EFS were evaluated using a paired $t$ test. $p < 0.05$ was considered significant.

## RESULTS

*Responses to metals in resting and MCh-contracted tracheal strips.* The chloride salts of the metals ($Ta^{5+}$, $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Cr^{2+}$, $Cr^{3+}$, and $Ni^{2+}$) were examined for their ability to cause contraction or relaxation of tracheal strips under basal force or contracted with MCh (Fig. 1). For every metal except $Co^{2+}$, cloudiness or a precipitate occurred at higher concentrations. Responses were judged to reflect solely the effect of a metal on the strip per se, as opposed to being the result of precipitation of the MKH solution, if they occurred in concentrations in which neither cloudiness nor a precipitate occurred.

In strips under basal force, $Co^{2+}$, $Cd^{2+}$, and $Ni^{2+}$evoked weak contractions ($>3 \times 10^{-6}$ M; 5–15% of the response to 120 mM KCl). At $10^{-3}$ M, $Co^{2+}$ relaxed the preparations. $Ta^{5+}$ and $Zn^{2+}$ caused weak relaxation responses, while neither $Cr^{2+}$nor $Cr^{3+}$ had any effect until the concentration ($10^{-4}$ M) just preceding the one causing cloudiness. Comparable results were obtained in MCh-contracted strips, with the only differences being that $Co^{2+}$ and $Ni^{2+}$ caused weak relaxations in low concentrations, and $Cd^{2+}$ did not elicit the weak contractions that were evident in the absence of MCh.

*Effect of metals on reactivity of tracheal strips to MCh.* The ability of the metals to alter reactivity of the strips to MCh was examined by comparing MCh concentration–response curves obtained in the absence or presence of the metals (Fig. 2). Both a low ($10^{-6}$ M) and a high ($3 \times 10^{-4}$ M) metal cation concentration was used. A precipitate or cloudiness of the MKH solution did not occur after the single addition of $3 \times 10^{-4}$ M metals to the bath. No metal in low concentration had any effect on the EC50 or maximum responses to MCh. $Ni^{2+}$ ($10^{-6}$ M) significantly potentiated responses to three concentrations of MCh, while the higher concentration ($3 \times 10^{-4}$ M) had an inhibitory effect at $3 \times 10^{-7}$ M MCh. In the presence of $3 \times 10^{-4}$ M $Cd^{2+}$, responses to MCh were inhibited, while $Cr^{2+}$ and $Ni^{2+}$ slightly inhibited responses to some concentrations of MCh.

*Effects of metals on responses to EFS.* We examined whether a low concentration ($10^{-6}$ M) of four of the metal cations ($Co^{2+}$, $Cd^{2+}$, $Cr^{3+}$, and $Ni^{2+}$) could affect neurogenic responses elicited with EFS. $Co^{2+}$ was selected because it had been observed to cause contraction of the strips (Fig. 1); however, this metal had no effect on responses to EFS ($n = 6$; data not shown). No metal had any effect on the eNANC contraction phase, when it was manifested in the responses (results not shown). Figures 3–5 depict the effects of $Cd^{2+}$, $Cr^{3+}$, and $Ni^{2+}$ on cholinergically mediated contractile and iNANC-mediated relaxant responses. $Cd^{2+}$ significantly inhibited contractile responses of preparations under basal force in the presence of indomethacin but had no effect on contractions nor on iNANC responses under the other conditions. An array of effects were produced by $Cr^{3+}$ (Fig. 4): the two greatest of these were the marked inhibition of iNANC-mediated relaxant responses ($\pm$MCh; Figs. 4B and 4E) in the absence of indomethacin and the inhibition of contractile responses of basal preparations when indomethacin was present (Fig. 4C). $Cd^{2+}$ had small but significant potentiating effects on contractile responses (Figs. 4A, 4D, and 4F) that appeared to depend on whether indomethacin was present, but the dependence of the effect on the presence of indomethacin was not clear. $Ni^{2+}$ was generally devoid of any effect on EFS-induced responses but did significantly inhibit contractile responses of preparations under basal tone (Fig. 5C).

In summary, three of the four metals, examined in a low concentration at which they had no direct effects (Fig. 1) and did not affect reactivity to MCh (Fig. 2), altered EFS-induced neurogenic responses.

## DISCUSSION

We examined the effects of the cations of seven metals found in HM alloys and observed that they have effects on the

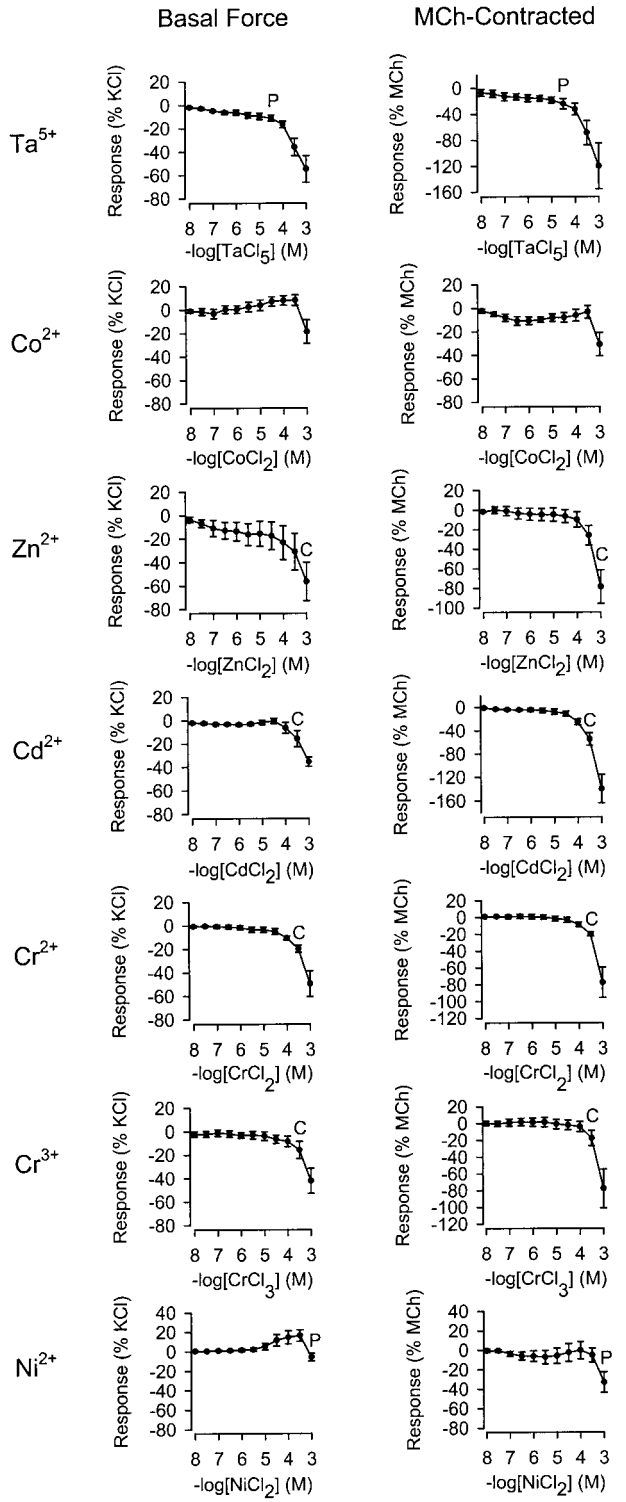


**FIG. 1.** Concentration–response curves for the chloride salts of $Ta^{5+}$, $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Cr^{2+}$, $Cr^{3+}$, and $Ni^{2+}$ in uncontracted tracheal strips under basal force (left) and in MCh($3 \times 10^{-7}$ M)-contracted strips (right). Responses of basal preparations were normalized with respect to the reference contraction to 120 mM KCl (% KCl); responses of MCh-contracted preparations were normalized with respect to the MCh-induced contraction (% MCh). Positive values denote contraction, and negative values indicate relaxation. $n = 6$ separate tracheas for each metal, with both conditions (i.e., ±MCh) studied in each trachea.

tone of airway smooth muscle, on reactivity to MCh, and on nerves innervating the smooth muscle of the guinea pig trachea. In pharmacological terms, the metals were not equivalent in their actions and they were moderately potent, giving rise to some effects in the micromolar range. The nature of some of the effects is such that they could affect airway diameter were they to occur *in vivo* following dissolution from HM particles.

Since Co is a major constituent of HM alloys, it was of interest that $Co^{2+}$, in low concentrations, caused contraction of the airway smooth muscle. This effect was shared by $Cd^{2+}$, to a lesser degree, and $Ni^{2+}$. These findings suggest that $Co^{2+}$ derived from inhaled HM could decrease airway diameter. When the metals are present in alloys, $Cd^{2+}$ and $Ni^{2+}$ might act synergistically with $Co^{2+}$ to give rise to a greater obstruction. In this context it is difficult to understand the significance of the relaxation to $Co^{2+}$ at high concentrations ($10^{-3}$ M); is there perhaps an intermediate concentration of $Co^{2+}$ that has no effect?

Experiments to identify possible interactions between the metals' effects, in the protocols used in this study, are logical to pursue. The number of possible metal combinations of two or more metals that could be explored is very large. The selection of relevant metal combinations, in concentration ratios that mimic those seen in alloys, could be guided by the establishment of a reference HM alloy. Such a reference standard does not exist, however, and a multiplicity of HM alloys is used in manufacturing.

Except for $Cd^{2+}$, no metal salt affected reactivity to MCh, either with respect to the EC50 of the agonist or the maximum response. $Ni^{2+}$ ($10^{-6}$ M) had a potentiating effect in selected low MCh concentrations. In the higher concentration ($3 \times 10^{-4}$ M) $Ni^{2+}$ inhibited responses to MCh slightly, and $Cd^{2+}$ caused a substantial inhibitory effect. To the degree that MCh is representative of other transmitters, neuropeptides, and mediators that contract airway smooth muscle, we conclude that, with the possible exception of $Ni^{2+}$, the response to these metals does not involve an alteration in reactivity of the airway smooth muscle in a direction that could lead to or exacerbate obstruction.

Our results indicate that the nerves that control airway diameter in the trachea are potential targets for the effects of some HM cations, i.e., $Cd^{2+}$, $Cr^{2+}$, and $Ni^{2+}$, but not $Co^{2+}$. Notably these effects occurred at a low concentration ($10^{-6}$ M) at which the metals had little or no other effects (Figs. 1 and 2). In the presence of indomethacin, which inhibits prostanoid formation, thereby reducing spontaneous tone which is characteristic of this *in vitro* preparation, cholinergically mediated contractile responses were inhibited by $Cd^{2+}$, $Cr^{3+}$, and $Ni^{2+}$. This effect could reflect a prejunctional inhibition of acetylcholine release by these metals and/or a postjunctional inhibition of the effects of released acetylcholine. In view of the results shown in Fig. 2, a prejunctional effect of $Cr^{3+}$ and $Ni^{2+}$ and a postjunctional effect of $Cd^{2+}$ is likely to have occurred. Inasmuch as EFS stimulates all nerve efferents, the reduction

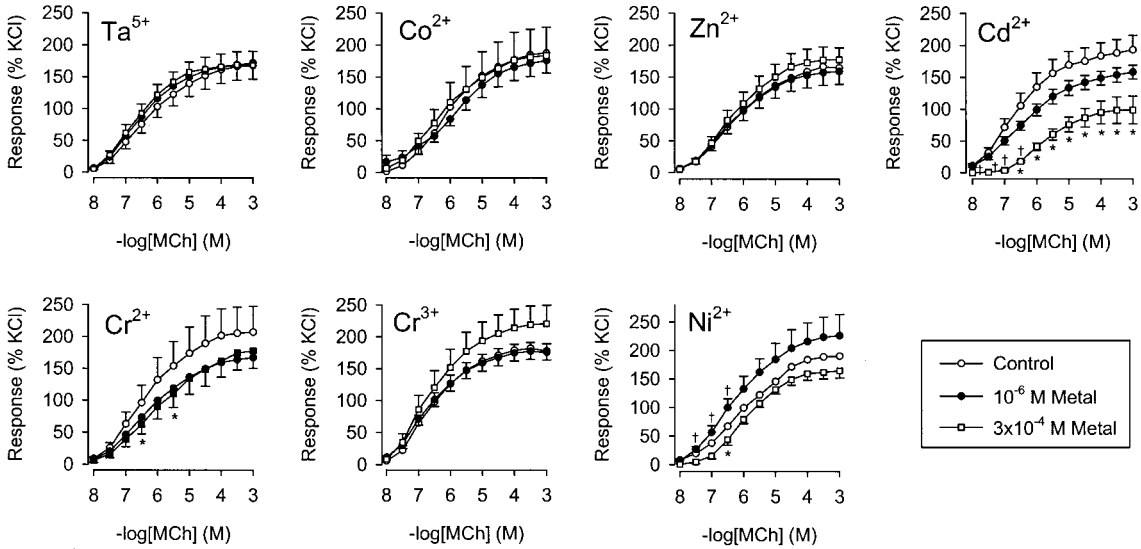


**FIG. 2.** Effect of the chloride salts of $Ta^{5+}$, $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Cr^{2+}$, $Cr^{3+}$, and $Ni^{2+}$ on MCh concentration–response curves of guinea pig tracheal strips. $n = 6$ separate tracheas for each metal, with three conditions (control and two metal concentrations) studied in each trachea. *$3 \times 10^{-4}$ M metal vs control, $p < 0.05$. †$10^{-6}$ M vs $3 \times 10^{-4}$ M, $p < 0.05$.

in the cholinergic contractions could have been due to an enhancement in the iNANC relaxation component of the response. However, neither $Cd^{2+}$ nor $Ni^{2+}$ had any effect on EFS-induced relaxation responses, which buttresses the view that cholinergic nerve endings were the target of the inhibitory effect of these two cations. We do not understand why the inhibitory effects of the metals on neurogenic contractions were seen only in the presence of indomethacin or why they were not observed in strips that had been precontracted with MCh in the absence or presence of indomethacin. There may be an aspect of the control of acetylcholine release that is sensitive to the metals, which is masked in the presence of prostanoids or MCh.

Only $Cr^{3+}$ had an effect on iNANC relaxation responses, and the metal inhibited the relaxation responses. This effect occurred both in basal and MCh-contracted preparations, but it was not evident in the presence of indomethacin. Inhibition of the iNANC responses may have contributed to the potentiation of cholinergic responses seen in basal and MCh-contracted strips (Figs. 4A and 4F). However, cholinergic contractions were not potentiated under other conditions [MCh-contracted, indomethacin absent (Figs. 4D and 4E)] when the iNANC relaxation responses were also inhibited, and cholinergic responses were potentiated when the iNANC relaxations were not inhibited (Figs. 4F and 4G); no consistent correlation appears to exist between the two effects. Were it to occur *in vivo*, inhibition of iNANC nerves by $Cr^{3+}$ could remove an important control mechanism for dilating airways. Thus, the fact that iNANC-induced relaxations were sensitive to $Cr^{3+}$ is an effect that is consistent with the etiology of occupational asthma after HM exposure. This effect is very similar to that observed in an animal model of asthma, in which it was reported that antigen challenge resulted in an impairment in iNANC-induced dilation responses of airways (Miura *et al.*, 1992). It is, again, interesting that $Co^{2+}$ had no effect on iNANC responses.

The mechanisms of action of the metals seen in this study were not investigated. However, some effects that could aid in understanding the results seen here have been reported in the literature. Many of the metals studied here have been reported to block $Ca^{2+}$ channels in airway (Sarria *et al.*, 1989) as well as in nonairway (Busselberg, 1995; Arispe *et al.*, 1996; Sundgren-Andersson and Johansson, 1998) tissues. For example, $Cd^{2+}$ inhibits the release of acetylcholine from neural tissues (Vickroy *et al.*, 1992; Callister and Sah, 1997) and from skeletal muscle end plates (Braga and Rowan, 1994), supporting a provisional explanation for inhibition of cholinergic contraction by this metal; however, $Co^{2+}$ also inhibited acetylcholine release from synaptosomes (Vickroy *et al.*, 1992) but had no effect on any of the neurogenic responses of tracheal strips. It is reasonable to suggest that inhibition of iNANC-induced relaxations by $Cr^{3+}$ might also have resulted from blockade of $Ca^{2+}$ entry into the nerve terminals. The failure of $Cd^{2+}$ and $Co^{2+}$ to affect contractions to MCh is surprising in view of the fact that these metals block $Ca^{2+}$ channels in canine tracheal smooth muscle (Kotlikoff, 1988); this could reflect a species difference in sensitivity to the metals. Nevertheless, Takemoto *et al.* (1998) observed that $10^{-4}$ M $Cd^{2+}$ inhibited carbachol-induced contractions in guinea pig tracheal muscle and concluded that this occurred because of blockade of $Ca^{2+}$ channels. A number of studies have shown that $Ni^{2+}$, generally used in millimolar concentrations, inhibits $Ca^{2+}$ channels in airway smooth muscle of several species (Amrani *et al.*, 1994; Yang *et al.*, 1994; Janssen, 1997; Madison *et al.*, 1998) and can

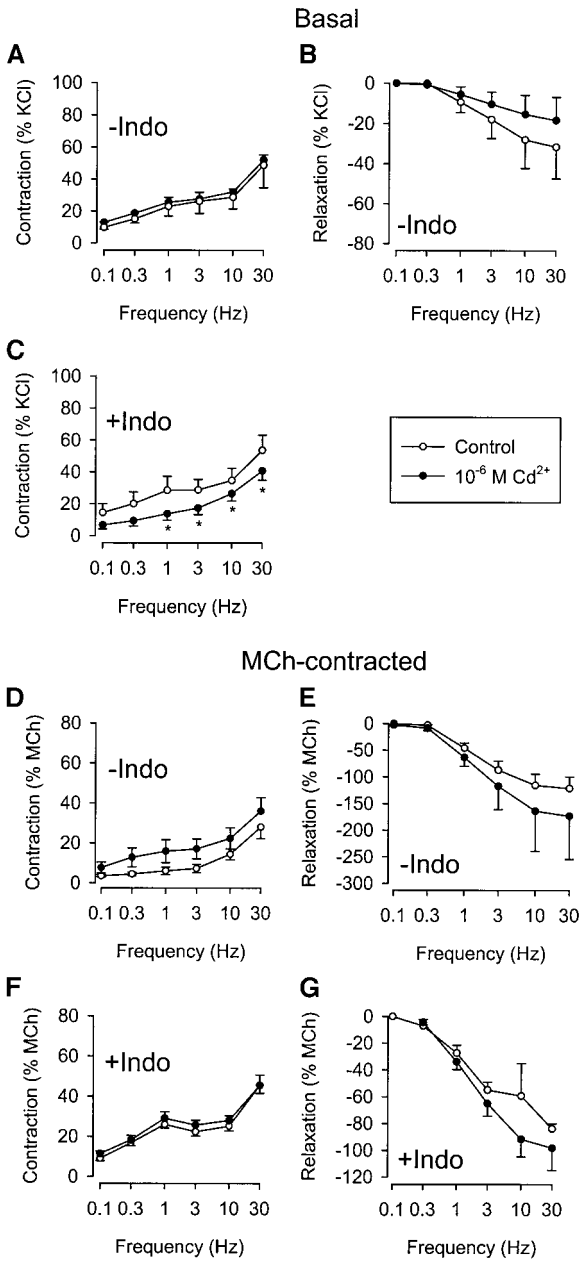


**FIG. 3.** Effect of $10^{-6}$ M $Cd^{2+}$ on EFS-induced contractile and relaxant responses of guinea pig tracheal strips under basal (A–C) and MCh ($3 \times 10^{-7}$ M)-induced (D–G) tone. The preparations were examined in the absence (A, B, D, and E) or presence (C, F, and G) of $3 \times 10^{-6}$ M indomethacin (Indo). Responses of preparations under basal force are normalized with respect to the reference contraction to 120 mM KCl (% KCl); responses of MCh-contracted preparations were normalized with respect to the MCh-induced contraction (% MCh). $n = 6$ separate experiments, with all conditions studied in each trachea. *$Cd^{2+}$ vs control, $p < 0.05$.

inhibit contractile responses (Cuthbert *et al.*, 1994). In contrast, we observed that $Ni^{2+}$ can elicit responses (Fig. 1) and had little if any effect on MCh-induced contractions, and we were unable to explain using published reports how $Co^{2+}$ and $Cd^{2+}$ could give rise to contractions by blocking $Ca^{2+}$ channels. We uncovered no studies of the effects of $Ta^{5+}$ on $Ca^{2+}$ channels.

Our findings can be applied to the understanding of the etiology of hard metal disease and occupational asthma in that we have identified potential targets in the lungs that could be

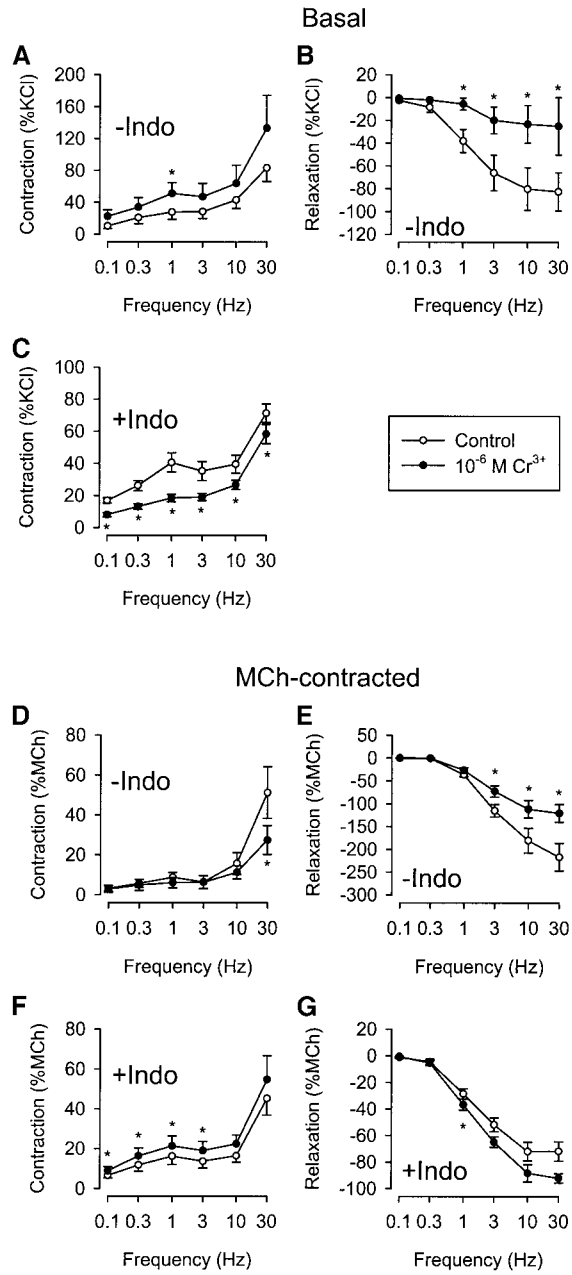


**FIG. 4.** Effect of $10^{-6}$ M $Cr^{3+}$ on EFS-induced contractile and relaxant responses of guinea pig tracheal strips under basal (A–C) and MCh ($3 \times 10^{-7}$ M)-induced (D–G) tone. The preparations were examined in the absence (A, B, D, and E) or presence (C, F, and G) of $3 \times 10^{-6}$ M indomethacin (Indo). Responses of preparations under basal force are normalized with respect to the reference contraction to 120 mM KCl (% KCl); responses of MCh-contracted preparations were normalized with respect to the MCh-induced contraction (% MCh). $n = 6$ separate experiments, with all conditions studied in each trachea. *$Cr^{3+}$ vs control, $p < 0.05$.

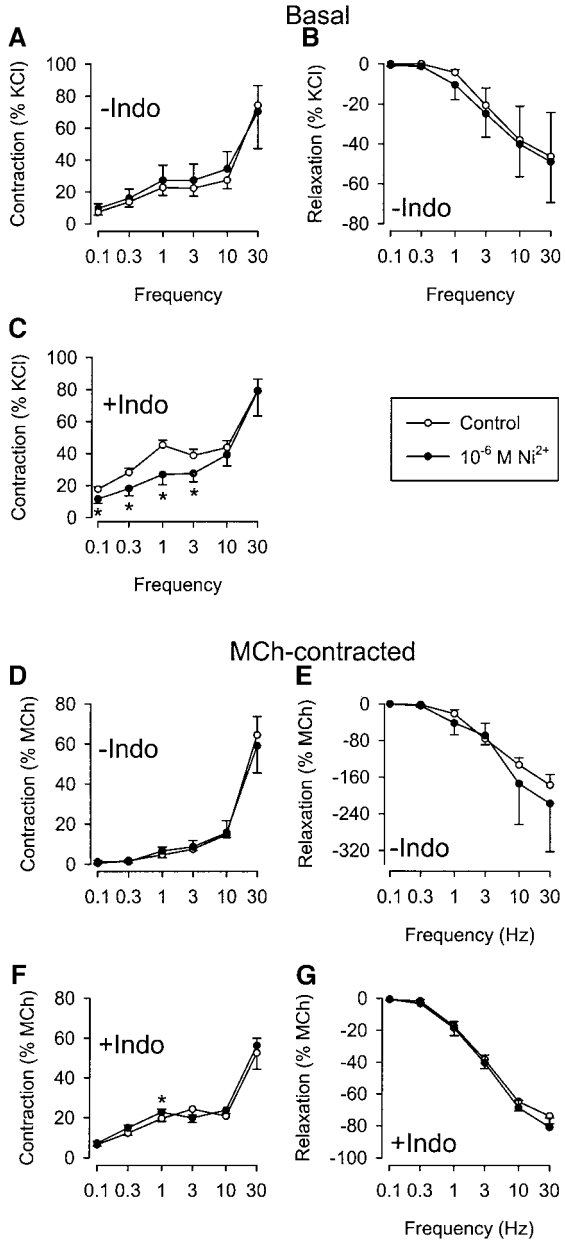


**FIG. 5.** Effect of $10^{-6}$ M $Ni^{2+}$ on EFS-induced contractile and relaxant responses of guinea pig tracheal strips under basal (A–C) and MCh ($3 \times 10^{-7}$ M)-induced (D–G) tone. The preparations were examined in the absence (A, B, D, and E) or presence (C, F, and G) of $3 \times 10^{-6}$ M indomethacin (Indo). Responses of preparations under basal force are normalized with respect to the reference contraction to 120 mM KCl (% KCl); responses of MCh-contracted preparations were normalized with respect to the MCh-induced contraction (% MCh). $n = 6$ separate experiments, with all conditions studied in each trachea. *$Ni^{2+}$ vs control, $p < 0.05$.

affected by soluble metals derived from inhaled HM particles. Clearly, our *in vitro* studies cannot model these complex clinical entities, and the relative contributions of the observed effects to the overall progression of the diseases is not known. We have observed effects of some metals in low, micromolar concentrations, which we postulate to be clinically relevant. We presume that the concentrations of dissolved metals in the lung are low, but actual values have not been reported, in either humans or animal models. In fact, due to the complexities of clearance processes, it is unlikely that the concentration of dissolved metals is uniform in the lung, as would be expected during the removal of a metal from an alloy particle's surface.

In conclusion, in order to understand how metals in HM alloys could contribute to the development of lung disease, we examined the effects of chloride salts of six metals on guinea pig tracheal strips. We studied their effects using three protocols that model ways by which airway diameter could be regulated. A single generalization cannot summarize the cumulative results of several metal-specific effects that were observed; however, some metals were capable of contracting the smooth muscle and interfering with excitatory and/or inhibitory neurotransmission. The metals have little influence on reactivity to MCh; with the exception of $Cd^{2+}$, the observed effects were not profound. None of the results can be interpreted on the basis of the known effects of these metals on NO production (see introduction).

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Mention of a brand name does not constitute product endorsement.

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