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Cadmium inhibits acid secretion in stimulated frog gastric mucosa

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ABSTRACT

Cadmium, a toxic environmental pollutant, affects the function of different organs such as lungs, liver and kidney. Less is known about its toxic effects on the gastric mucosa. The aim of this study was to investigate the mechanisms by which cadmium impacts on the physiology of gastric mucosa. To this end, intact amphibian mucosae were mounted in Ussing chambers and the rate of acid secretion, short circuit current (I_{sc}), transepithelial potential (V_t) and resistance (R_t) were recorded in the continuous presence of cadmium. Addition of cadmium (20 μM to 1 mM) on the serosal but not luminal side of the mucosae resulted in inhibition of acid secretion and increase in NPPB-sensitive, chloride-dependent short circuit current. Remarkably, cadmium exerted its effects only on histamine-stimulated tissues. Experiments with TPEN, a cell-permeant chelator for heavy metals, showed that cadmium acts from the intracellular side of the acid secreting cells. Furthermore, cadmium-induced inhibition of acid secretion and increase in I_{sc} cannot be explained by an action on: 1) H_2 histamine receptor, 2) Ca^{2+} signalling 3) adenylyl cyclase or 4) carbonic anhydrase. Conversely, cadmium was ineffective in the presence of the H^+/K^+-ATPase blocker omeprazole suggesting that the two compounds likely act on the same target. Our findings suggest that cadmium affects the functionality of histamine-stimulated gastric mucosa by inhibiting the H^+/K^+-ATPase from the intracellular side. These data shed new light on the toxic effect of this dangerous environmental pollutant and may result in new avenues for therapeutic intervention in acute and chronic intoxication.

Introduction

Cadmium is a highly toxic, non-essential heavy metal that displays significant threats to living organisms by interacting with vital cellular functions. The effects induced by acute and/or chronic exposure of target organs and processes (kidneys, liver, lungs, cardiovascular, immune and reproductive systems) to cytotoxic concentrations of cadmium have been intensely investigated (Jarup and Akesson, 2009). These studies defined diverse mechanisms of cadmium uptake, accumulation and toxicity that strongly depend on cell type and condition/duration of exposure.

Gastrointestinal ingestion through contaminated food and drinking water is considered to be the main source of cadmium for non-occupationally exposed, non-smokers (Jarup and Akesson, 2009). Gastric mucosa is therefore the epithelium that is first exposed to food and water containing high levels of cadmium. Previous studies concerning the chronic effect of cadmium on rat gastric mucosa showed that a 30 day exposure leads to significant accumulation of the heavy metal in the mucosa and significant reduction in the basal acid output (Asar et al., 2000; Oner et al., 1994). According to Oner et al. the resulting lipid peroxidation would bring about decreased production of gastric mucin and PGE_2 and impairment of endogenous protective mechanisms (Oner et al., 1994). Asar et al. suggest that the significant decline in the recorded basal acid output in response to cadmium may be explained by a loss of parietal cells population (Asar et al., 2000). Moreover, Dupuy and Szabo showed that rats pre-treated with cadmium (2.5 mg/100 g body weight) for 30 min presented a significant inhibition of gastric acid output after 1 h of piloric ligation (Dupuy and Szabo, 1986). These studies were all performed in whole stomach ex vivo preparations where the gastric acid output was measured after titration of the extracted luminal fluid. Therefore, no information regarding the potential effects of acute exposure to this heavy metal on the cellular mechanisms leading to gastric acid secretion is available.

Acid secretion by gastric oxyntopeptic/parietal cells is an intricate process that is tightly regulated by paracrine, endocrine and neural factors. Stimulation of acid secretion typically involves an initial elevation in cAMP followed by activation of a cAMP-dependent protein kinase (PKA) that triggers the translocation and insertion of the gastric proton pump (H^+/K^+-ATPase) together with K^+ and Cl^- conductances into the apical plasma membrane of acid secreting cells. The process ultimately produces net output of protons, chloride ions and water across the apical plasma membrane of these cells (Yao and Forte, 2003; Gerbino et al., 2007).

Abbreviations: I_{sc}, short circuit current; V_t, transepithelial potential; R_t, transepithelial resistance; CA, carbonic anhydrase; AC, adenylyl cyclase.

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According to available literature cadmium might theoretically affect gastric acid secretion at several different levels: *i*) cadmium can interfere with the activity of different plasma membrane receptors (Coddou et al., 2005; Giridhar et al., 1992; Stoica et al., 2000), hence the heavy metal might interact with the histamine receptor (H_2) and/or with transporters located on the basolateral membrane and involved in acid secretion; *ii*) it can interfere with essential intracellular messenger cascades, Ca^{2+} and cAMP signalling, (see (Thevenod, 2009); *iii*) cadmium is known to inhibit carbonic anhydrase by displacing key metal components such as zinc from the proper binding site, thus it may affect the secretory processes that depend on HCO_3^- availability in the gastric mucosa; and *iv*) it could potentially block gastric (H^+/K^+)-ATPase activity. Regarding the latter possibility, it has already been shown that cadmium impacts on the activity of most members of this class of P-ATPases (Hechtenberg and Beyersmann, 1991; Herak-Kramberger et al., 2000; Kinne-Saffran et al., 1993; Verbost et al., 1988; Zhang et al., 1990) including the gastric proton pump (Hongo et al., 1990).

The purpose of this paper was to determine the mechanism by which micromolar concentrations (ranging from 20 to 100 μM) of cadmium can affect acid secretion in amphibian gastric mucosa. Although plasma cadmium levels in the general population are typically less than 0.02 μM, we used this range of concentrations because significantly higher levels are found in smokers and people with occupational exposure (Tsalev and Zaprianov, 1984).

Here, by using a classic, straightforward electrophysiological technique on a well characterized experimental model such as the intact amphibian gastric mucosa (Gerbino et al., 2007; Caroppo et al., 2004, 2001), we found that exposure to serosal cadmium produces inhibition of histamine-stimulated acid secretion. This in turn causes an elevation of short circuit current, which is correlated with the electrogenic Cl^- flux known to be regulated by the proton pump (Reenstra et al., 1987).

Materials and methods

Tissue preparation and solutions. Experiments were performed on gastric fundus mucosa of *Rana esculenta* in accordance with the Italian guidelines for animal experiments. The frogs were kept in an aquarium at room temperature and killed by decapitation and destruction of the spinal cord. The stomach was isolated and the muscle layer and connective tissue were removed by blunt dissection. Where needed, tissues were kept in resting state by adding 100 μM cimetidine. To stimulate acid secretion a maximal concentration of histamine (500 μM) was added to the serosal solution and maximal rate of acid secretion was reached within 60–90 min. After stable secretion values were reached, cadmium (20–100 μM) and/or other drugs were added to the serosal and/or luminal solution. All chemicals were of reagent grade and purchased from Farmitalia Carlo Erba (Milan, Italy), Sigma and Fluka Chemie AG (Buchs, Switzerland), Alexis Biochemicals (Lausen, Switzerland).

Acid secretion measurements. Tissues were mounted vertically between two halves of a Lucite chamber having an exposed area of 0.64 cm^2. Each half-chamber consisted of a circular fluid canal of 2.5 ml total volume filled with modified Ringer's solution that was constantly re-circulated by means of a bubble lift. The control Ringer's solution on the serosal side contained (in mM): 102.4 Na^+, 4.0 K^+, 1.4 Ca^{2+}, 0.8 Mg^{2+}, 91.4 Cl^-, 17.8 HCO_3^- and 11 D-glucose. Solutions were gassed continually with 5% CO_2 in O_2 (pH 7.36).

The luminal solution was unbuffered and had the following composition (in mM): 102.4 Na^+, 4.0 K^+, 91.4 Cl^-, 15 isethionate, 7 mannitol, and 11 D-glucose. To prevent accumulation of CO_2, this solution was gassed with 100% O_2 that was passed through a bottle containing $Ba(OH)_2$ solution (50 mM). Acid secretion was measured with the pH-stat method (Radiometer, Copenhagen, Denmark). The titration procedure was activated every 10 min using 5 mM NaOH to titrate the mucosal solution to a constant pH of 5.50. The transepithelial potential (V_t) was continuously monitored with a voltmeter using two calomel half-cells connected to each bath solution *via* agar-Ringer's bridges. V_t is reported as that of the mucosal solution with respect to the serosal side and the short circuit current (I_{sc}) was measured using a voltage clamp device as originally described by Ussing and Zerahn (1951). The transepithelial resistance (R_t) was calculated using the open circuit potential difference that developed 1 s after brief interruption of constant short-circuiting conditions.

Data analysis and statistics. Mean values are expressed ± S.E.M. of n individual experiment performed. The significance of the observations was evaluated by Student's *t* test for paired or unpaired data as appropriate and $p<0.05$ denoted a statistical difference.

Results

Serosal but not luminal cadmium inhibits histamine-stimulated acid secretion

Our study was performed on amphibian gastric mucosa. This epithelium has been used as a model for the study of the function of the gastric mucosa in many laboratories, and its secretory and ion transport properties have already been very well characterized (Carlisle et al., 1978; Debellis et al., 1990, 1992, 1998; Forte et al., 1967; Kasbekar and Durbin, 1965; Machen and McLennan, 1980; Ruiz et al., 1993; Shoemaker and Sachs, 1972; Silen et al., 1975; Supplisson et al., 1991).

Ingestion of contaminated food or water is one of the main causes of cadmium intoxication. Due to the extremely low intra-luminal pH recorded in stimulated stomach, it is reasonable to assume that the apical side of the gastric mucosa can be exposed to cadmium ions derived by degradation of Cd–protein complexes (with metallothionein or glutathione) induced by acidic pH. Therefore, we first evaluated the effect of exposure to luminal cadmium in epithelia stimulated with high histamine concentrations (500 μM). The rate of acid secretion, measured at 10 min intervals, reached a steady value usually 60–90 min after stimulation and was constant up to 2 h (data not shown). When $CdCl_2$ was added to the Ringer's solution perfusing the luminal side of histamine-stimulated mucosae, the rate of acid secretion did not change significantly even after prolonged treatment (2 h) at all the concentrations used (Fig. 1A, 20–100 μM and 1 mM, respectively).

Since it is well established that chronic cadmium intoxication results in increased blood levels of this heavy metal (Tsalev and Zaprianov, 1984; Asar et al., 2000) we next assessed the effect of Cd^{2+} addition to the serosal side of the epithelium. These experiments, summarized in Fig. 1B, showed that basolateral incubation of stimulated mucosa with cadmium (20, 50 and 100 μM) resulted in a significant, irreversible (data not shown), inhibition of acid secretion. While the final inhibitory effect (i.e. the final value of acid secretion rate reached after addition of cadmium) was similar at all the concentrations used, the effect of 100 μM cadmium was faster. It started earlier and reached ~70% of maximal inhibition two times faster than the other concentrations used (40 min vs 80 min). As shown in Table 1, the cadmium-induced decrease in acid secretion rate was associated to significant elevations in potential difference (V_t) and transepithelial resistance (R_t). The increase in R_t might reasonably exclude harmful effects of cadmium on cell–cell adhesions (tight junctions) reported by others (Prozialeck and Niewenhuis, 1991; Prozialeck et al., 1993).

The lack of effect of cadmium from the apical side of the epithelium may be explained by one or more of these factors: 1) low permeability of the apical membrane, likely more tight to cadmium than the basolateral