Effects of mercuric ions on isolated guinea-pig ileum

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The possible physiological significance of low concentrations of Hg^{2+} in the guinea-pig ileum has been investigated. Responses to nerve stimulation by single electrical shocks, acetylcholine (ACh), and histamine (Hi) and the response to ACh of a depolarized preparation were examined. A stimulant effect of Hg^{2+}, in the concentration range of 10 nM–1 μM, dominated in intestine accommodated in biological saline solution. This excitatory effect was probably mainly due to stimulation of a depolarization-coupled initiation of the contraction. The inhibitory effect of Hg^{2+}, 10 nM–1 μM, observed in depolarized muscle was presumably due to a decreased Ca^{2+} availability for the contractile process. In the higher concentration range, 1–100 μM, a probably direct action on the contractile elements causing irreversible deterioration of the preparation seems to be present. □ *Amalgams; cholinergic transmission; corrosion; excitable tissue; smooth muscle*

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The cytotoxicity of amalgam and its corrosion products has been demonstrated in human cell culture studies (1, 2). Clinical manifestations such as allergy and oral lichen planus in conjunction with amalgam restorations have been reported (3–6). Allergy to mercury is common (26%) in patients with oral lichen planus (6). Furthermore, the presence of dental alloys may cause pain in the oral mucous membranes and a metallic taste (7–9). It is possible that extraneous agents in diminutive amounts, in the environment of nerves, muscles, or sensory organs, could alter the response of stimuli (10). In the present study special interest will be paid to whether the Hg^{2+} released from amalgams in the oral cavity could exert effects on excitable tissue.

Mercury is the main constituent (about 50%) of dental amalgam. Mercury could be released from amalgam restorations, as fine alloy particles or as mercuric ion (11), due to wear and corrosion. Vaporization of mercury from the surface of amalgam restorations may also occur (12). Exposure to mercury vapor and amalgam dust also occurs when amalgam restorations are removed by means of rotating instruments.

In vivo investigations have shown increased concentrations of corrosion products from dental alloys in the hard and soft tissues of the mouth (13, 14). The concentration of mercury in gingiva in contact with amalgam was 19–380 μg/g of tissue, as compared with control values of 0–10 μg/g (15). Increased levels of blood mercury have been reported in persons with amalgam restorations compared with a group without amalgams (12). The blood mercury level in the amalgam group was 3.5 nM (range, 0–16.5; $n = 47$) and in the control group 1.5 nM (range, 0–5; $n = 14$). However, there are other reports showing no correlation between blood mercury level and the presence of amalgam restorations (16). Elevated mercury concentrations have also been registered in the dental pulp after insertion of amalgam in cavities (344.5 μg/g wet weight) (17).

The mercury that may be released from amalgams is present in the body mainly in the divalent state. Attention has earlier been paid to the biological effect of mercuric salts in connection with their use in the treatment of syphilis and as mercurial diuretics (18). The affinity of mercury for sulfur and sulfhydryl groups is a major factor behind the biochemical properties of mercury and mercury compounds (19).

Mercury has been considered to be of

special significance in causing pathological neural and/or muscular signs. The manifestations in vivo of massive chronic mercury poisoning are often conspicuous. It is known that high concentrations of methyl-Hg (5 μM) in vivo can exert toxic actions on excitable tissue (20). However, during distribution of small amounts of mercury, effects that are less pronounced than those associated with acute and massive chronic poisoning could be exerted on tissues in the body. Hg^{2+}, in micromolar concentrations, has been shown in vitro to exert actions on isolated heart (21) and smooth-muscle contractions (22), neuromuscular (23–28), synaptic (29, 30), and sensory (31) transmission, Ca^{2+} release from sarcoplasmic reticulum (32), respiration and metabolism in brain tissue (33), activity of brain enzymes (34), and other activities vital for the function of excitable tissues (35, 36). However, it is not quite clear whether or how lower concentrations, <1 μM, of mercuric salts influence the physiologic functions of excitable tissues.

It was therefore decided to investigate to what extent effects of Hg^{2+}, in concentrations lower than those usually studied—that is, <1 μM—could be elicited on excitable tissue. This concentration is close to the detection limit of Hg^{2+} in biological tissue and is also more significant with regard to the possible exposure to Hg^{2+} in dentistry. The guinea pig ileum has earlier been shown to be a suitable, convenient, and sensible model system when studying the effects of low concentrations of heavy metals on excitable tissue (37). Some preliminary observations have been reported earlier (38).

Materials and methods

Mottled, male or female guinea-pigs (400–700 g) were killed by a blow on the head. A segment of 2–5 cm was taken from the distal part of the guinea-pig ileum and placed in a 50-ml organ bath containing Tyrode's solution aerated with 6.5% CO_2 in O_2 and kept at 37°. The Tyrode solution had the following composition: 136.7 mM NaCl, 2.7 mM KCl, 11.9 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.3 mM $NaHPO_4$ (all analytical grade; E. Merck, Darmstadt, FRG) and 5.6 mM glucose (BDH Chemicals Ltd, Poole, UK) dissolved in deionized water. The pH was 7.4 and was not changed during the experiments by the additions of Hg^{2+} salts. Depolarization of the muscle was achieved by replacing the NaCl with equimolar amounts of KCl. When Ca^{2+} contraction of depolarized ileum was studied, $CaCl_2$ was replaced by equiosmolar amounts of KCl.

After being mounted, the muscle was allowed to equilibrate for at least 1 h and was slightly stretched (2–5 mN) before the experiment started. Contractions were recorded isometrically by a force-displacement transducer (Grass FT 03C) coupled to a polygraph (Grass model 7B).

Single electrical shocks of 0.5 msec were given every 60 sec with a strength (0.2–1.5 V) giving about half the maximum response. The neurogenic nature of the response was verified by its sensitivity to tetrodotoxin (0.3 μM). Acetylcholine (ACh) was added in concentrations of 50 nM–10 μM to non-depolarized and 5–100 μM to depolarized tissue. The test concentrations were selected to give about half the maximum response. Histamine (Hi) was added in a similar manner to non-depolarized tissue (0.1–30 μM). Drugs were added every 5th min, followed by washing after 45 sec. In the $CaCl_2$-free organ bath solution, $CaCl_2$ (10 mM) was added every 12 min to induce a contraction and was allowed to act for 2 min. After the washing, EDTA (1 mM) was added for 5 min to deplete Ca^{2+} from the preparation (39).

Added $HgCl_2$ was dissolved in bath medium to avoid dilution effects and added to the organ bath in a volume of 0.2–1.0 ml. At the start of testing, $HgCl_2$ was added to a concentration of 0.1–10 nM, and after that the $HgCl_2$ concentration in the organ bath was repeatedly 10-folded by new additions.

The effects of $HgCl_2$ were also tested when Ca^{2+} blockers, verapamil (5 μM) and sodium nitroprusside (sodium nitroferricyanide, $C_5FeN_6Na_2O$, 10 or 20 μM), were present in the organ bath.

The effects of hyperosmolarity were exam-

ined by comparing equiosmolar concentrations of $HgCl_2$ and NaCl. Preparations with high spontaneous activity were excluded from the study. Each type of experiment was performed on 5–20 preparations from 3–15 guinea pigs. Usually two preparations were taken from each guinea-pig.

Drugs and salts

Acetylcholine chloride (Hoffmann-La Roche & Co. AG, Basel, Switzerland), histamine chloride (Apoteksbolaget AB, Stockholm, Sweden), verapamil (Isoptin® hydrochloride; Knoll AG Chemische Fabriken, Ludwigshafen, FRG), sodium nitroprusside, $HgCl_2$ (E. Merck), and tetrodotoxin (Sigma Chemicals, St. Louis, Mo., USA).

Results

When the guinea-pig ileum was stimulated neurogenically, no clear effects of Hg^{2+} were detected in concentrations below 10 nM. At higher concentrations of Hg^{2+} (range, 10 nM–1 μM), the response to electrical field stimulation was an increased peak tension, at 1 μM often combined with a slight rise in the 'resting tone' of the preparation (Fig. 1). At 10 nM an effect was seen in about 10% of the preparations, at 100 nM in about 30%, and at 1 μM a clear effect was seen in 65% of the preparations. This excitatory effect could not be reproduced in the same preparation unless the Hg^{2+} concentration was increased by a power of 10–100. $Hg(NO_3)_2$ had the same effects as $HgCl_2$. No effects were observed when equiosmolar or equimolar concentrations of NaCl were added.

Reduction of the $CaCl_2$ concentration in the bath by 50% increased the sensitivity to Hg^{2+} (range, 10 nM–1 μM), but the increased peak tension and tonic response became shorter in time.

The response to ACh and Hi in ordinary Tyrode's solution could be divided into a rapid phase of contraction (1–10 sec) and a slow one persisting throughout the drug exposure. The peak tension of the rapid phase was greater than that of the slow. Hg^{2+}, at a range of 10 nM–1 μM, enhanced the contractile response to ACh and Hi, but the effect of Hg^{2+} was seen only on the rapid phase (Fig. 2). The increase in tension was counteracted by Ca^{2+} (1 mM) (Fig. 3). The excitatory effect of Hg^{2+} on ACh- and Hi-induced contractions was often slighter than that exerted on contractions due to field stimulation.

Higher concentrations (range, 1–10 μM) of Hg^{2+} initially increased the response to the stimuli (electrical, ACh, and Hi). However, a 2- to 10-min exposure to these concentrations gradually depressed the responses. At concentrations of 10–100 μM the intestines became virtually insensitive to stimuli.

The response to ACh and Hi in depolarized tissue was decreased but not altered with regard to the shape of contractions—that is,

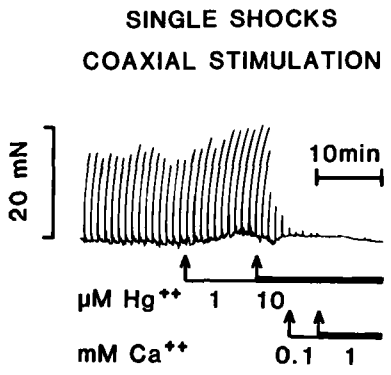

Fig. 1. Effects of Hg^{2+} on field-stimulated guinea-pig ileum. Hg^{2+}, 1 μM, enhanced the response, whereas it was impaired by a higher concentration. Increases of the Ca^{2+} concentration did not counteract this depressant effect.

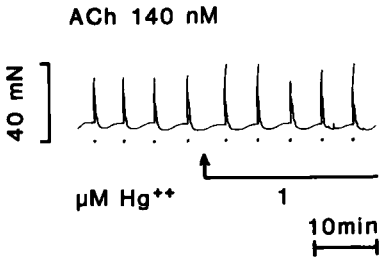

Fig. 2. Effect of Hg^{2+} on ACh-induced contractions. A low dose increased or prolonged the response.

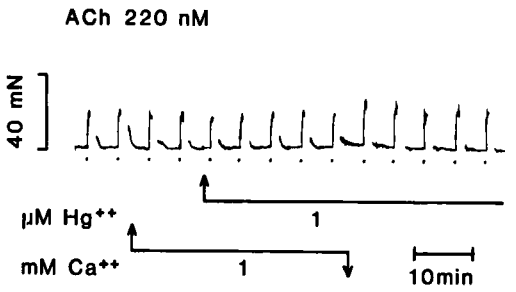

Fig. 3. Effects of Hg^{2+} on ACh-induced contraction. The augmentative effect of Hg^{2+} was counteracted by increasing the Ca^{2+} concentration of the Tyrode's solution (see Fig. 2).

one rapid phase of short duration and one slow phase of long duration could still be separated. The relative difference in strength of the phases was about the same as in the non-depolarized state. Both phases of contraction induced by ACh and Hi in depolarized tissue were depressed by Hg^{2+} (range, 10 nM–100 μM) (Fig. 4). At 10–100 μM complete extinction of the response to ACh was seen. Ca^{2+}, 1–10 mM, did not counteract this depression.

The Ca^{2+} blockers verapamil and sodium nitroprusside blocked the stimulant action of Hg^{2+} (range, 10 nM–1 μM) during ACh-induced contractions. At high concentrations of Hg^{2+} (> 1 μM) the Ca^{2+} blockers did not counteract the depressant effect of Hg^{2+}.

In depolarized and EDTA-treated tissue the response to added $CaCl_2$ (10 mM) was also divided into two phases, one short and

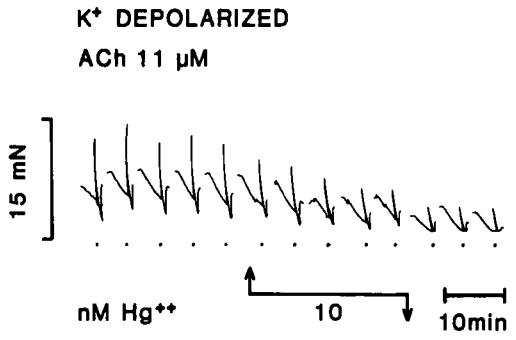

Fig. 4. Effect of Hg^{2+} on ACh-induced contraction in depolarized tissue. The response was depressed by a very low concentration of Hg^{2+} (see Figs. 1–3).

one slow, long-lasting contraction, which was often stronger than the short one. Hg^{2+}, in a range of 10 nM–100 μM, decreased the strength of both phases without changing the difference between them.

Discussion

The guinea pig ileum is a frequently used model system for studying drug and ion effects on the autonomic neuromuscular transmission. A previous study (37) showed that Cu^{2+} in very low concentrations (range, 10 nM–1 μM) had both stimulatant and inhibitory effects at different levels in the neuromuscular transmission of the guinea pig ileum. The present study showed that similar effects were exerted by Hg^{2+} in the same concentration range. However, the effects exerted by Hg^{2+} seemed to be more prominent than those exerted by Cu^{2+} (37).

The known actions of ACh on isolated intestinal smooth muscle were summarized in a previous paper (37). ACh affects the contractile response of the muscle cell by inducing changes in the membrane potential, by firing of action potentials, and also in depolarized muscle by increasing intracellular Ca^{2+}. In accordance with the earlier study (37) it is likely that the facilitating effect of Hg^{2+}, in the low concentration range (10 nM–1 μM), on the smooth-muscle contractions was mainly exerted via a step in the neuromuscular transmission which involves depolarization of the muscle cell membrane. Hg^{2+}, 40 μM, has earlier, in the sciatic nerve-sartorius muscle preparation of frogs, been shown to produce a rapid depolarization in the initial stage of exposure, accompanied with spontaneous discharges of action potentials which lead to twitches of individual fibers (25). However, since the facilitating effect of Hg^{2+} on neurogenically induced contractions appeared to be stronger than those due to stimulation with ACh or Hi, a presynaptic action of Hg^{2+} may also be present. Hg^{2+} has earlier been shown to reduce the permeability of the nerve cell membrane (23), and Hg^{2+}, >100 nM, has been shown to increase evoked transmitter release in the neuromuscular junction of the

frog (24, 26). The exact mechanism of this latter effect of Hg^{2+} in submicromolar concentrations is not clear, but direct actions of Hg^{2+} on the intracellular mechanisms regulating transmitter release seem likely (25).

A rise in the 'resting tone' of the preparation was observed at somewhat lower concentrations for Hg^{2+}, 1 μM, than for Cu^{2+} (37), but, as with Cu^{2+}, Hg^{2+} in itself increased the resting tone of the preparation. Heavy metals have been suggested to increase the calcium permeability of the sarcoplasmic reticulum in vesicles derived from rabbit fast skeletal muscle (32). The capacity of heavy metals to form or initiate formation of disulfide groups from sulfhydryl groups was proposed as an explanation of the increased leakage of Ca^{2+} through the sarcoplasmic reticulum membrane.

In accordance with earlier findings (37) the Ca^{2+} blockers verapamil and sodium nitroprusside blocked the stimulant action exerted by low concentrations (10 nM–1 μM) of Hg^{2+}. This suggests that the postsynaptic stimulant action of Hg^{2+} was mediated via increased Ca^{2+} availability for the contractile process. The experiments provided no clear hint at any specific site of action, although the tachyphylaxis of the Hg^{2+} effect suggests an action on either a Ca^{2+} store that is easily depleted or an easily deteriorated Ca^{2+} transport mechanism.

In depolarized muscle all concentrations of Hg^{2+} tested (range, 10 nM–100 μM) diminished contractions induced by ACh and Ca^{2+}-induced contractions in Ca^{2+}-depleted muscle. The antagonistic effect of Hg^{2+} on the Ca^{2+} contracture suggests an interference with Ca^{2+} influx into the cell. It is possible that this depressant action of Hg^{2+} was also present in non-depolarized tissue but in the low concentration range (10 nM–1 μM) masked by the stronger stimulant actions mentioned above. In higher concentrations (1–100 μM) the effect of Hg^{2+} differed from that of Cu^{2+} (37), which in this high concentration range initially had a stimulant effect on ACh-induced contractions in depolarized tissue. This points to an action of Hg^{2+}, stronger than that of Cu^{2+}, directed towards the contractile elements of the muscle. High concentrations of Hg^{2+}, >1 μM, have earlier been reported to exert inhibitory effects on both pre- and post-synaptic nerve terminal sites (23–27, 29).

In the concentrations usually studied, >1 μM, clear effects of Hg^{2+} are often recorded. In concentrations below 1 μM the effects on excitable tissue are less frequent. However, the present study shows that even submicromolar concentrations of Hg^{2+} could exert clear effects on excitable tissue in an isolated organ.

The effect of an element will be dependent on both concentration and state, ionic or bound, at the possible site of action. At present we do not know the fraction of free Hg^{2+} ions or the proteins to which Hg^{2+} is bound after corrosion in the oral cavity of man. The binding of corroded metallic ions to 'salivary-type proteins' has been shown in vitro to vary with the amount, composition, and pH of the artificial saliva (40). A decreased salivation could increase the concentration of free ion in the saliva and also increase the penetration into the mucosa (41). Changes in the oral epithelium could change the conditions for penetration of elements into the mucosa. Penetration into non-keratinizing epithelium is greater than into keratinized epithelium. Loading or mechanical wear of dental restorations could alter the barrier function and permeability of the oral mucosa (42). It is also possible that the excitability and threshold level in sensory receptors could be altered with changed osmotic conditions. The possible higher blood mercury levels of amalgam wearers reported (12) have been supposed to originate from inhalation of Hg^{o} vapor released from the restorations. An initial accumulation of mercury has been shown in the erythrocytes after inhalation of Hg^{o} (0.1 ng/ml) (43). The lung cells and erythrocytes are probably the main sites for oxidation of mercury vapor in the body. It is not known to which extent Hg^{2+} bound to proteins in the blood stream could exert actions on excitable tissue when distributed to target organs such as the brain, myocardium, spinal ganglions, nerves, and eye (44).

With in vitro experiments performed in a protein-free nutrition solution, the concentration of free ions is fairly constant.

However, many effects exerted in vitro may not appear in vivo owing to regulatory systems in the organism keeping the concentrations of externally supplied ions low. Many factors determine the dose-response relationship, and the effects also often have a threshold dose (45). Since the factors influencing the interindividual variation in threshold dose often are not quite clear, extrapolation of dose-response relationships to low doses and low response rates is difficult.

Finally, it should be emphasized that irreversible actions are characteristic of Hg^{2+} on excitable tissues (21, 23–28, 30). The potent neurotoxicity of Hg^{2+} as compared with other heavy metals is probably due to its very tight binding to SH groups (the stability constant of HgS is about 10^{-50}) combined with a relatively large access to the intracellular compartment of the tissues (10). This irreversible and accumulating feature of Hg^{2+} could make it a special potential hazard in the excitable tissues, especially if buffering, chelating, and other protecting systems in the tissues are impaired.

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