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Mercury deposits in neurons of the trigeminal ganglia after insertion of dental amalgam in rats

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An amalgam filling was inserted into the first upper molar of 12 rats and the animals were killed after 3–9 months. Tissue sections from the trigeminal ganglia and the brain stem were then investigated with a sensitive histochemical technique to trace mercury deposits. Within the trigeminal ganglia, nerve cells with mercury deposits were observed in seven out of 12 rats, whereas no mercury was detected in sections from the brain stem. The mechanism responsible for the accumulation of mercury in neurons of the trigeminal ganglia is discussed.

Keywords: mercury, trigeminal ganglion, tooth pulp

Introduction

Minor amounts of mercury vapor are released from dental amalgam restorations, especially during mastication (Svare *et al.* 1987). The concentration of mercury in the tooth pulp of teeth with amalgam fillings has been reported to be higher than in teeth without amalgam (Schiele *et al.* 1987). According to Lentz and *et al.* (1989), the pulpal tissue of dogs will be exposed to mercury when amalgam is placed in deep, unlined preparations. In a previous study in rats, we observed retrograde axonal transport of mercury in the ipsilateral trigeminal nerve after injection of an aqueous solution of mercuric chloride into the coronal pulp of the first upper molar (Arvidson & Arvidsson 1990). The aim of the present investigation was to ascertain whether mercury released from dental amalgam accumulates in neurons of the trigeminal ganglion by retrograde transport from the dental pulp. For this purpose, an amalgam filling was inserted unilaterally into the first upper molar in rats, and sections from the trigeminal ganglia were investigated with a histochemical technique (Hacker *et al.* 1988) to trace mercury deposits. In some animals, sections from the brain stem, including the central projections of the trigeminal nerve, were also investigated.

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Materials and methods

The experiments were performed on 14 female Sprague-Dawley rats. The rats were housed in plastic and steel cages in an ambient temperature of 20 °C. The animals were fed a standard laboratory diet and had free access to drinking water. Under general anesthesia (chloral hydrate 30 mg per 100 g body weight, i.p.), the pulp cavity of the first upper molar on the left side was exposed with a dental drill and an amalgam filling (Dispersalloy; Johnson & Johnson, East Windsor, NJ) was inserted in the cavity, no cavity liner being used. According to the producer, this amalgam is composed of 50% mercury, and 50% alloy powder containing 70% silver, 16% tin, 13% copper and 1% zinc.

The animals were reanesthetized and killed 3–9 months after the insertion of the amalgam filling. All rats were perfused through the ascending aorta first with saline and then with a fixative consisting of 3% glutaraldehyde in 0.1 mol l^{-1} phosphate buffer. After perfusion, both trigeminal ganglia were removed and immersed in fixative for 3 h. In six rats killed 9 months after insertion of amalgam, the brainstem was also removed for investigation. Two control rats were included.

The samples were immersed in 30% sucrose until saturated. Serial frozen horizontal sections with a thickness of 30 μm were cut from both trigeminal ganglia. In rats killed 9 months after amalgam insertion, frozen sections were also cut from different levels of the brain stem.

The sections were mounted on glass slides which were immersed in a 0.5% gelatin solution and dried before

development. The slides were then placed vertically in a glass container filled with developer for the demonstration of mercury by silver acetate autometallography (Hacker *et al.* 1988). The developer was prepared by mixing two solutions, A and B. Solution A was composed of 100 mg of silver acetate (Fluka Chemie AG, Switzerland) dissolved in 50 ml of distilled water; and solution B was composed of 500 mg of hydroquinone (Fluka AG) dissolved in 50 ml of citrate buffer, with 25 ml of gum arabic solution added. The sections were developed in the dark for 70 min at 22 °C. The slides were then washed in running tap water at 40 °C for 45 min and immersed for 10 min in a 5% sodium thiosulfate solution. Finally, the slides were dehydrated in alcohol and xylol, mounted in Eukitt and viewed unstained.

To ascertain whether the deposits represented mercury, some of the sections from mercury-exposed mice were exposed to potassium cyanide, according to Danscher & Rungby (1989). Following this treatment, the only possible catalysts for the autometallographic reaction are mercury sulfides and metallic gold.

Results

The results are summarized in Table 1. In five rats, no traces of mercury were observed in either ganglion. In three rats, a small number of labeled neurons were restricted to the left ganglion (ipsilateral to the amalgam insertion) and in one rat a few labeled neurons were observed only in the right ganglion. In three rats, labeled neurons were found in both ganglia and the total number of neurons with mercury deposits was considerably greater than in the other animals. The mercury deposits in neurons had a granular appearance (Figure 1). Sections from the brain stem did not show any mercury deposits in neurons, axons or in the parenchyma.

Table 1. Experimental plan

Rat	Survival after amalgam insertion (months)	No. of nerve cells with mercury deposits		Brain stem
		left TG	right TG	
1	3	0	0	NI
2	3	6	0	NI
3	3	0	0	NI
4	6	0	0	NI
5	6	91	65	NI
6	6	4	0	NI
7	9	0	0	—
8	9	97	35	—
9	9	199	83	—
10	9	0	14	—
11	9	7	0	—
12	9	0	0	—

An amalgam filling was inserted into the first upper molar on the left side and the rats were killed after 3–9 months. TG, trigeminal ganglion; NI, not investigated; —, no mercury deposits present.

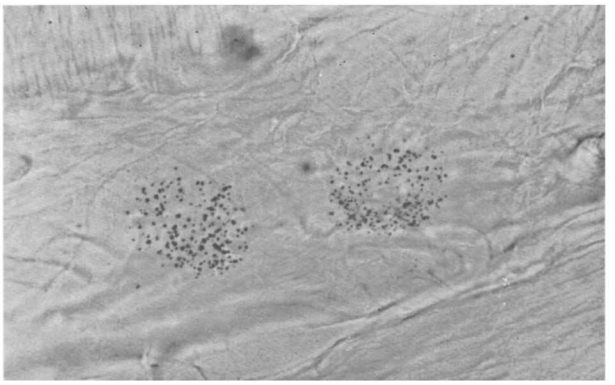

Figure 1. An unstained section (30 μm thick) from the trigeminal ganglion of a rat killed 9 months after amalgam insertion. Mercury deposits are seen as black granules in two nerve cells.

Sections from control rats without amalgam manifested no mercury deposits in any of the tissue sections.

The pattern and the amount of deposits were the same in untreated sections and those incubated with cyanide. As the animals were not exposed to gold, and as no deposits were observed in sections from control animals, all the staining must stem from mercury sulfides.

Discussion

Animal experiments have shown mercury released from dental amalgam to be present in tissue sections from other regions of the body. After subcutaneous implantation of powdered dental amalgam in Guinea pigs, Eley & Cox (1986) observed mercury deposits in the cytoplasm and nuclei of proximal tubular cells of the kidney. Danscher *et al.* (1990) reported deposits of mercury in several organs including the kidneys, spinal ganglia and the anterior pituitary 1 year after insertion of dental amalgam in Vervet monkeys.

Mercury released from dental amalgam in humans has been demonstrated in adjacent oral tissues such as the gingiva (Fredén *et al.* 1974), dentin (Massler & Barber 1953, Söremark *et al.* 1962), dental pulp (Möller 1963, Schiele *et al.* 1987), tooth roots and surrounding alveolar bone (Till & Maly 1978). Hørsted-Bindslev *et al.* (1991) reported transport of mercury from dental amalgam into dentinal tubules in Vervet monkeys; mercury was also found in pulpal cells.

In the present study, no mercury deposits were observed in neurons of the trigeminal ganglia in five of 12 rats. The seven remaining rats manifested great individual variation in the number of neurons with mercury deposits, though the reason for this variation is obscure. One important factor might be individual differences in the amount of mercury released from the amalgam filling. Studies in humans have shown that the concentration of mercury in blood and urine may vary greatly between individuals with the same number of amalgam surfaces (Åkesson *et al.* 1991). Although the release of mercury from dental amalgam fillings may be increased by bruxism (Hogan *et*

al. 1991; Sällsten *et al.* 1991), in the present study we observed no signs of bruxism among the rats with amalgam. In some rats, labeled nerve cells were observed also in the ganglion contralateral to the insertion of amalgam, which suggests a hematogenous distribution of mercury to the ganglia. If the mechanism was retrograde transport, only neurons in the ipsilateral ganglion would be labeled, since the sensory innervation of teeth is strictly unilateral (Arvidsson 1975, Marfurt & Turner 1984). Retrograde tracing with horseradish peroxidase (HRP) has demonstrated the neurons innervating the first upper molar in the rat to be located within a restricted region of the ganglion (Marfurt & Turner 1984, Fried *et al.* 1989). In the present study, neurons labeled with mercury deposits were in part localized outside this region, a finding inconsistent with retrograde transport from the dental pulp as the sole mechanism for the accumulation of mercury in nerve cells.

The blood vessels of the trigeminal ganglia in rodents are fenestrated and highly permeable (Arvidson *et al.* 1973). Large molecules such as HRP and ferritin which have passed from the capillaries into the extracellular space are taken up by endocytosis into nerve cells (Rosenbluth & Wissig 1964, Arvidson 1979). In blood, inorganic mercury is bound to hemoglobin in erythrocytes and to plasma proteins (Berlin 1986). It is reasonable to assume that mercury released from dental amalgam is taken up into nerve cells of the trigeminal ganglia by endocytosis, after leakage of the protein-bound metal from ganglionic vessels. However, the results of the present study do not exclude that retrograde transport of mercury may occur in addition to a direct uptake into nerve cells. Some of the neurons labeled with mercury were localized within the region corresponding to the sensory innervation of the pulp of the first upper molar; and in animals where neurons in both ganglia were labeled, the number of labeled neurons was considerably greater on the side ipsilateral to the amalgam. The exact position of the deep part of the amalgam filling within the tooth might determine whether uptake of mercury into nerve endings of the pulp occurs.

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