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Metabolism-Dependent Toxicity of Methimazole in the Olfactory Nasal Mucosa

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Abstract: In mice given a single intraperitoneal injection of the antithyroid drug methimazole (0.44 mmol/kg; 50 mg/kg) detachment of the olfactory neuroepithelium and necrosis of the Bowman' glands in the lamina propria was observed 24 hr after administration. Three days after administration there was an atypical epithelium throughout the olfactory region and the Bowman's glands had disappeared. Pretreatment with the olfactory cytochrome P450 inhibitor metyrapone protected against the methimazole-induced changes at this site. In mice injected with the methimazole analogues 1-methylimidazole or 4-methylimidazole (0.44 mmol/kg; 36 mg/kg) or the antithyroid drug propylthiuracil (0.22 mmol/kg; 38 mg/kg) no morphological changes were observed in the olfactory mucosa. The results suggest that methimazole-induced toxicity in the olfactory mucosa is related to metabolism-dependent changes of the thiol group.

Methimazole and other derivatives of thiourea depress thyroid function by irreversibly inhibiting the thyroid peroxidase needed for the synthesis of thyroid hormones (Engler *et al.* 1982). Methimazole is also an inhibitor of other peroxidases, cytochrome P450-dependent monooxygenases (P450), cysteine conjugate S-oxidase and flavin-containing monooxygenase (Hunter & Neal 1975; Sausen & Elfarra 1990; Bandyopadhyay *et al.* 1993). Pretreatment with methimazole prevents the hepatic bioactivation and toxicity of thiobenzamide, most likely via inhibition of flavin-containing monooxygenase (Chieli & Malvadi 1983). The potent toxicity of the 2,6-chlorinated thiobenzamide analogue, chlorthiamid, in the olfactory mucosa was recently reported (Brittebo *et al.* 1991) and the effects of methimazole and some P450-inhibitors on the chlorthiamid-induced toxicity in the mouse olfactory mucosa were therefore examined. The studies showed that methimazole did not decrease, but increased, the chlorthiamid-induced toxicity in the olfactory mucosa (unpublished observation). In the methimazole-injected control mice marked morphological changes in the olfactory mucosa were noted. Since methimazole has been reported to affect the sense of smell in humans (Schiffman 1983) it was considered of interest to further examine the methimazole-induced changes at this site. In this communication the effects of methimazole, two methimazole analogues and the antithyroid drug propylthiouracil, on the mouse olfactory mucosa are reported. In addition, the effects of an olfactory cytochrome P450-inhibitor on the methimazole-induced toxicity have also been examined.

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Materials and Methods

Methimazole (1-methylimidazole-2-thiol), propylthiouracil and metyrapone were obtained from Sigma Chem. Co., St. Louis, MO, U.S.A. 1-Methylimidazole and 4-methylimidazole were obtained from Aldrich-Chemie, Steinheim, Germany.

Female NMRI-mice (20–30 g) and C57B1/6 mice (28 g) were purchase from B & K Universal (Sollentuna, Sweden). The animals were housed in a temperature-controlled room at 22° with a 12/12 hr light/dark cycle and given a standard pelleted diet (Ewos AB, Södertälje, Sweden) and tap water *ad libitum*. In the first experiment, C57B1/6 mice (n=3) were given a single intraperitoneal injection of methimazole (0.44 mmol/kg; 50 mg/kg dissolved in saline; 5 µl/g body wt.) or saline and were killed by exposure to gaseous CO_2 24 hr later. In the later experiments, only NMRI mice were used. Groups of 3 mice were given a single intraperitoneal injection of methimazole (0.5, 5.0, 25 or 50 mg/kg) as above and were killed 24 hr later. Additional mice were injected with methimazole (50 mg/kg) and were killed 4 hr (n=3) or 3 days (n=3) later. In order to inhibit the olfactory P-450 enzyme activities (Brandt *et al.* 1990) mice were given metyrapone (100 mg/kg intraperitoneally; dissolved in saline) 20 min. before the injection of a toxic dose of methimazole (50 mg/kg) as above. Metyrapone-treatment will not induce any morphological changes in the olfactory mucosa (Brandt *et al.* 1990). Finally, NMRI mice were injected intraperitoneally with propylthiuracil (0.22 mmol/kg; 38 mg/kg; dissolved in saline; 10 µl/g body wt.; n=3), 1-methylimidazole or 4-methylimidazole (0.44 mmol/kg; 36 mg/kg; dissolved in saline; 5 µl/g body wt.; n=3) or saline (n=4) and were killed 24 hr later. The entire nasal regions and pieces of the livers were dissected, fixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7), embedded in methacrylate resin and processed for histopathology as described previously (Brittebo *et al.* 1991). Transversal sections (2–3 µm) from the nasal regions (serial sections; >10 glass slides/mouse) were cut and stained with haematoxylin/periodic acid-Schiff or eosin or with toluidine blue. The livers were embedded in paraffin, sectioned (2 glass slides/mouse) and stained with haematoxylin/eosin.

Results and Discussion

The results of this study showed that methimazole was a potent toxicant in the olfactory mucosa following a single

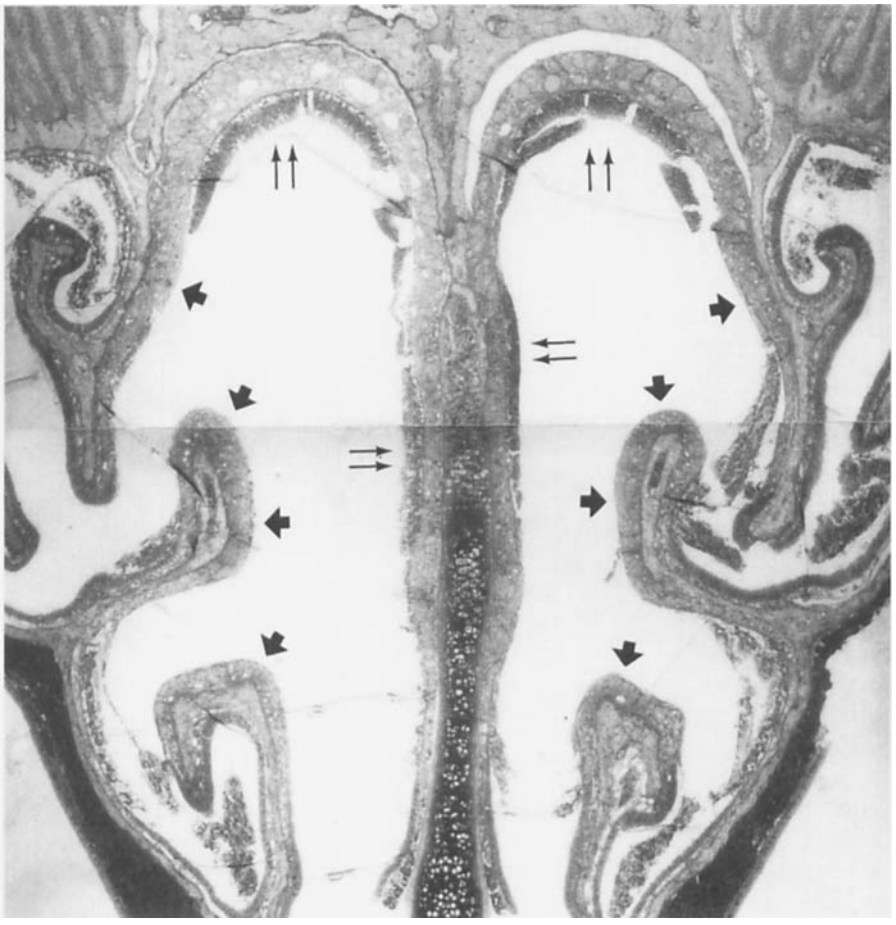

Fig. 1. Section through the anterior olfactory region of a NMRI mouse killed 24 hr after an intraperitoneal injection of methimazole (50 mg/kg). The neuroepithelium has disappeared completely in the lateral parts and on the turbinates leaving a denuded basal membrane (thick arrows). Areas with detaching neuroepithelium are present on the nasal septum and in the dorsal region (thin arrows). Toluidine blue-stained tissue section. Original magnification ×50.

intraperitoneal injection in mice. As soon as 4 hr following administration of 50 mg/kg the neuroepithelium showed signs of detachment preferentially in the lateral parts of the olfactory region and on the tips of the turbinates. Throughout the olfactory region a decreased periodic acid-Schiff-staining intensity in the contents of the Bowman's glands was observed and the acinar cells appeared to separate leading to an enlarged lumen. Absence of periodic acid-Schiff-stainable glycoproteins in the Bowman's glands has previously been observed in mice given subtoxic doses of the olfactory toxicants dichlobenil and chlorthiamid (Brittebo *et al.* 1991 & 1992) suggesting that a decreased periodic acid-Schiff-staining intensity in these glands may be a sensitive and early marker for a disturbed function.

Twenty-four hr after administration of methimazole the neuroepithelium in the lateral parts of the olfactory region and on the tips of the turbinates had disappeared leaving a denuded basal membrane with scattered basal cells (fig. 1 and 2). In the dorsal parts of the olfactory region the neuroepithelium showed signs of detachment above the basal cell layer (fig. 1 and 2). The Bowman's glands were necrotic or showed degenerative changes and lack of periodic acid-Schiff-staining throughout the olfactory region whereas the nerves and vessels showed no morphological changes (fig. 3B). The methimazole-induced changes in the olfactory mucosa of NMRI or C57Bl mice were similar (fig. 1 and 2). Three days after a single toxic dose of methimazole, the neuroepithelium was replaced with an atypical non-ciliated epithelium throughout the olfactory region (fig. 3D). The Bowman's glands had disappeared leaving only the connective tissue and a few scattered periodic acid-Schiff-negative acini whereas no morphological changes were observed in the nerves and vessels (fig. 3D). No toxic effects were observed in other parts of the nasal region or in the liver and no clinical signs of toxicity were noted in the methimazole-treated mice. In the vehicle-treated mice the nasal mucosae had a normal appearance and the Bowman's glands showed an intense red periodic acid-Schiff-staining.

In methimazole-treated mice given 25 mg/kg and killed 24 hr later the morphological changes in the olfactory mucosa were less marked. The most prominent changes still occurred in the lateral parts of the olfactory region and on the tips of the turbinates (fig. 3A). In these areas the olfactory neuroepithelium showed signs of detachment above the basal cell layer. Throughout the olfactory region the periodic acid-Schiff-staining intensity in the Bowman's glands was absent or decreased, however. In mice given lower doses of methimazole (0.5 or 5.0 mg/kg) and killed 24 hr later the olfactory mucosa appeared normal.

Previous studies have shown that methimazole is metabolized by hepatic P450 and flavine-containing monooxygenase to reactive intermediates which become covalently

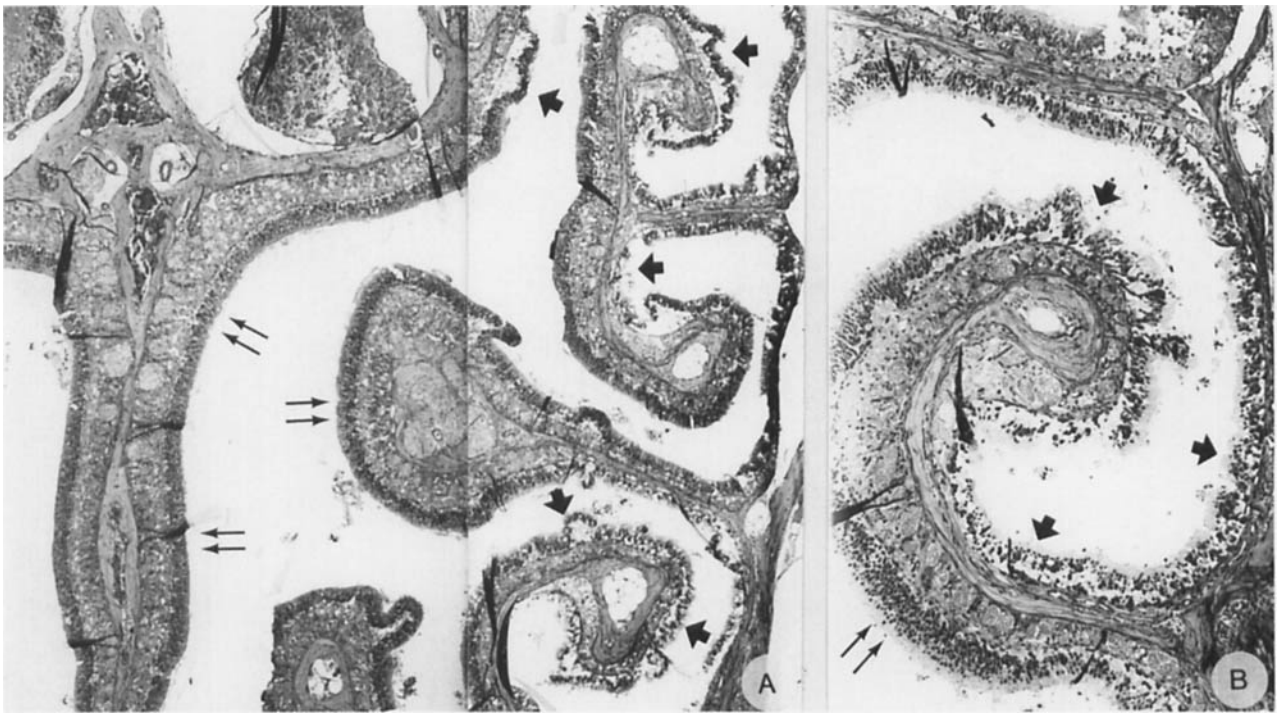

Fig. 2. (A) Section through parts of the posterior olfactory region of a C57B1 mouse killed 24 hr after intraperitoneal injection of methimazole (50 mg/kg). (B) Detail of an ethmoturbinate. In the lateral parts and on the ethmoturbinates (thick arrows) marked detachment of neuroepithelium is seen, whereas in the dorsal meatus and nasal septum the detachment of neuroepithelium is less pronounced (thin arrows). Toluidine blue-stained tissue sections. Original magnification (A) ×55; (B) ×110.

bound to tissue macromolecules (Poulsen *et al.* 1974; Lee & Neal 1978; Kedderis & Rickert 1985; Taurog *et al.* 1989; Decker & Doerge 1992). Pretreatment with the olfactory P450-inhibitor, metyrapone, completely protected against the methimazole-induced necrosis in the olfactory mucosa (fig. 3C) and periodic acid-Schiff-positive material could be noted in the contents of the Bowman's glands. Hence the methimazole-induced toxicity seems to be related to an enzyme-catalyzed metabolism of methimazole in the olfactory mucosa. Metyrapone-treatment will also inhibit the metabolic activation and toxicity of dichlobenil in the olfactory mucosa (Brandt *et al.* 1990; Eriksson & Brittebo 1991). The identity and localization of the methimazole- and dichlobenil-activating enzyme in the olfactory mucosa is probably not similar, however, because the initial sites of methimazole- and dichlobenil-induced toxicity in the olfactory region do not coincide. While the first methimazole-induced changes occurred in the lateral parts of the olfactory region, the dichlobenil-induced changes occur in the dorsomedial region. It should also be noted that dichlobenil is a more potent toxicant when approximately equimolar doses of dichlobenil (0.29 mmol/kg; 50 mg/kg) and methimazole (0.22 mmol/kg; 25 mg/kg) are compared (Brandt *et al.* 1990).

In liver methimazole is stepwise metabolized to the corresponding sulfenic and sulfinic acids with a concurrent formation of reactive intermediates (Poulsen *et al.* 1974). In mice given the methimazole analogues 1-methylimidazole or 4-methylimidazole, which are devoid of a thiol group, or the thyroid inhibitor propylthiuracil, no morphological changes were observed in the olfactory mucosa or in the liver. Thus the thiol group in methimazole seems to be important for the methimazole-induced toxicity, suggesting that enzyme-catalyzed changes of the thiol group will give rise to intermediates toxic to the olfactory mucosa.

In conclusion this study has identified a new site of metabolism-dependent toxicity of methimazole, the olfactory mucosa. Further studies on the mechanism of methimazole-induced toxicity in the olfactory mucosa are in progress.

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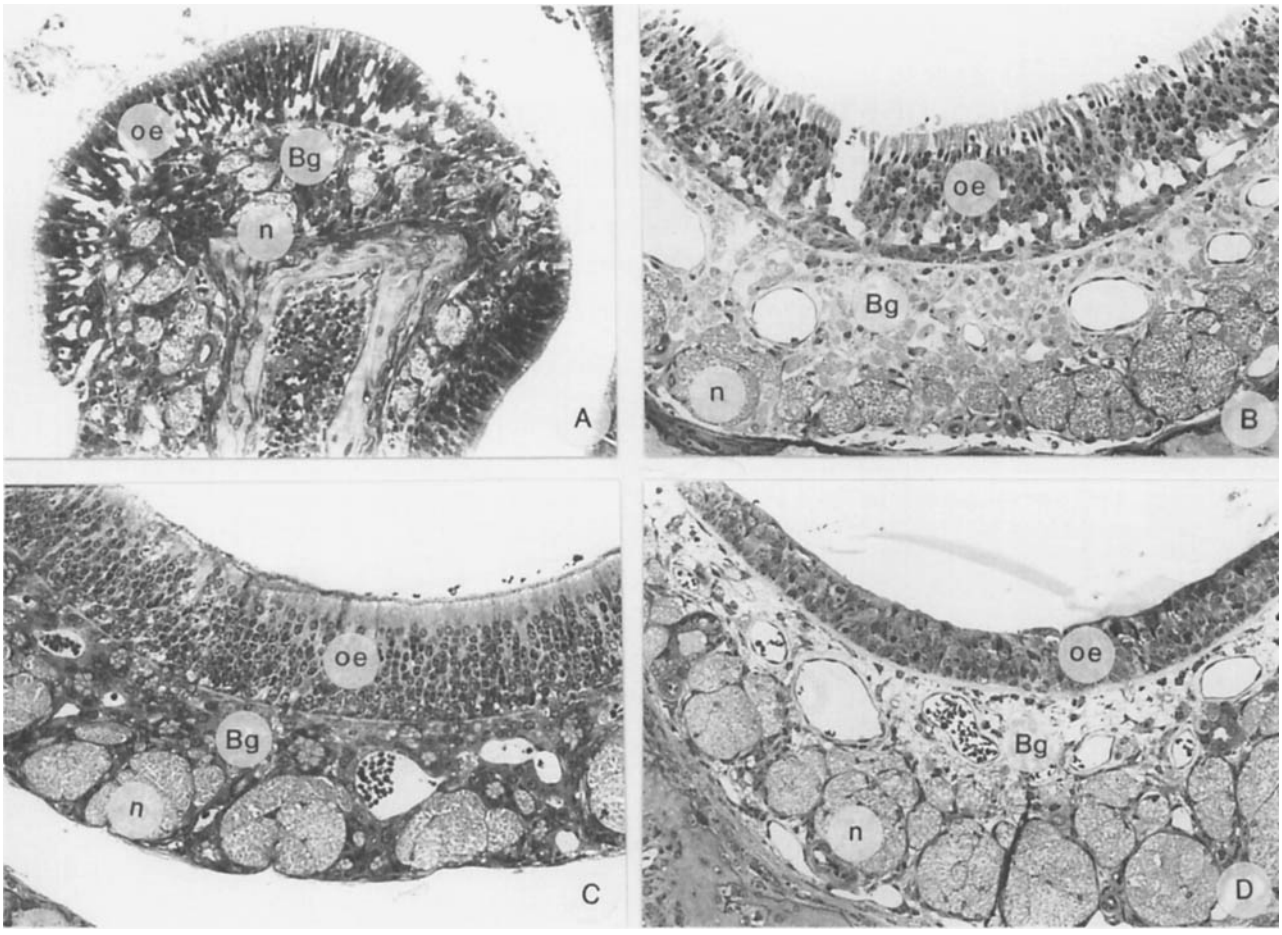

Fib. 3. (A) Section through the tip of a turbinate of a NMRI mouse killed 24 hr after intraperitoneal injection of methimazole (25 mg/kg). (B) Section through the dorsal meatus of a NMRI mouse killed 24 hr after intraperitoneal injection of methimazole (50 mg/kg). (C) Section through the dorsal meatus of a metyrapone-pretreated NMRI mouse killed 24 hr after intraperitoneal injection of methimazole (50 mg/kg). (D) Section through the dorsal meatus of a NMRI mouse killed 3 days after intraperitoneal injection of methimazole (50 mg/kg). A and B: The olfactory neuroepithelium of the methimazole-treated mice show signs of detachment above the basal cells. B: The Bowman's glands have disappeared completely in the methimazole-treated mouse. C: The olfactory neuropithelium and Bowman's glands are intact in the metyrapone-pretreated mouse given methimazole. D: The olfactory neuropithelium is atypical and the Bowman's glands have disappeared leaving only the connective tissue in the methimazole-treated mouse. The blood vessels and nerves are intact in all mice. (oe olfactory epithelium; Bg Bowman's glands; n nerve bundle; toluidine blue-stained tissue sections). Original magnification ×210.

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