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Retronasal Smell and Detection Thresholds of Iron and Copper Salts

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Abstract

Iron and copper salts, when placed in the mouth, may give rise to odorous compounds which complicate their functioning as chemical stimuli. The contribution of retronasal smell to perception of these metal salts at threshold has not been determined. Detection thresholds of the sulfate and chloride salts of ferrous iron and copper, and sodium chloride (as a control) were determined using a modified forced-choice ascending method of limits, with and without nasal occlusion. Threshold values were calculated from geometric means of individual estimates, and from interpolation on logistic regression and percent correct plots. Nasal occlusion raised thresholds for iron salts and copper but not sodium. The geometric mean detection thresholds with the nose open were 30, 64, 7.8, and 8.2 μM for $FeSO_4$, $FeCl_2$, $CuSO_4$, $CuCl_2$, respectively but rose to 160, 227, 24.6 and 15.6 with the nose closed. Metal salts of both iron and copper create a retronasally perceived olfactory stimulus at low concentration levels, probably arising from lipid oxidation products generated in the mouth.

Keywords

Taste thresholds; nasal occlusion; retronasal smell; metallic taste

1. Introduction

Several studies have measured detection thresholds of iron and copper compounds as shown in Table 1 [1-8]. The studies varied in terms of test methods, nasal occlusion condition, aqueous sample volumes, water types and statistical methods of analysis, all of which can affect the resulting threshold values [9]. In these studies, the condition of nasal occlusion was either not stated, or rarely used. If there is an effect of nasal occlusion on the thresholds the primary method of discernment of the compounds may be olfactory rather than gustatory in nature. The contribution of a retronasal smell would be indicated by a nasal occlusion effect. Retronasal smells are effectively eliminated by closing the nose during stimulus

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sampling [10] or by injecting a pure airstream through the external nares, preventing retronasal transport [11].

Ferrous salts are typically sensed as metallic, astringent and/or sweet at low concentrations and bitter or sour at high concentrations depending on their anions and concentrations [1, 12]. Odors in the mouth can cause reports of tastes, primarily due to passage of volatiles into the nasal passages from the mouth through the nasopharynx, called retronasal smell. The metallic sensation from ferrous sulfate solutions is primarily a retronasally perceived sensation as it is effectively decreased by nasal occlusion [12, 13]. Of the studies in Table 1, the use of nasal occlusion appears to increase threshold values. However, Lim and Lawless [14] reported that subjects could discriminate between ferrous sulfate solutions and water without a retronasal cue if the concentration is high enough (0.005M and 0.05M). Thus the contribution of olfaction to the perception of these complex stimuli remains unclear.

Salts of divalent metals such as iron, copper and zinc have different tactile, gustatory and olfactory components, some of which can be influenced by nasal occlusion [13 - 15]. $CuSO_4$ has some bitter and astringent properties and a metallic taste that is not consistently reduced by nasal closure. Lawless et al. [12] found a reduction in copper sulfate metallic taste with nasal closure in one study, but no reduction in a second condition when the metallic taste was defined more specifically for subjects as the sensation arising from ferrous sulfate. This could imply a lessened retronasal component from copper salts or a different origin of this metallic quality. Tactile sensations were produced on a non-gustatory surface (between the upper lip and gum) by $CuSO_4$ but less effectively so by $FeSO_4$ [14], suggesting a stronger astringency component to copper salts. The emergence of different sensory qualities may be concentration-dependent [12].

The objective of this study was to assess detection thresholds of ferrous sulfate and chloride, copper sulfate and chloride and sodium chloride (as a control), with and without nasal occlusion to determine (in a direct within-subjects comparison) if there is a contribution of retronasal smell to the detection of these substances at low concentrations.

2. Materials and Methods

2.1 Subjects

Three different groups of twenty healthy subjects (eight males for iron, seven males for copper and eight males for sodium, all between the ages of 18-65) with no reported taste problems or anosmias, from the Cornell University community in Ithaca, NY, volunteered to participate. All subjects were untrained and naïve to the hypothesis of the study. Informed consent was given before the test and compensation was provided at the conclusion. The research protocol was approved by the University Committee on Human Subjects.

2.2 Stimuli

The iron, copper and sodium compounds used in this study were ferrous sulfate ($FeSO_4$ • $7H_20$, Mallinckrodt Baker, Inc., Phillipsburg, NJ), ferrous chloride ($FeCl_2$ • $4H_20$, Mallinckrodt Baker, Inc., Phillipsburg, NJ), cupric sulfate ($CuSO_4$ • $5H_20$, EMD Pharmaceuticals, Durham, NC), cupric chloride ($CuCl_2$ • $2H_20$, EMD Pharmaceuticals, Durham, NC) and sodium chloride (NaCl, Mallinckrodt Baker, Inc., Phillipsburg, NJ). All stimuli were reagent grade and were prepared by dissolving the compounds in deionized water. Concentrations ranges were as follows: Iron sulfate and chloride, 3.28 μM to 12.5 mM, copper sulfate and chloride, 1.31 μM to 2 mM and sodium chloride, 614 μM to 375 mM. Dilutions were made by a factor of 2.5. Solutions were prepared every 3 hours during testing to prevent any by-products from oxidation [16]. The solutions were presented as 20 mL samples in 2 oz odorless plastic cups (Solo 2 oz Plastic Cups, Solo Cup Company, Urbana, IL) labeled with three-digit random numbers, and at room temperature (approximately 21 °C). All of the stimuli were sipped and expectorated.

2.3 Procedure

Iron, copper and sodium salts were tested by separate groups. For iron and copper, each group of subjects participated in four sessions, one for each anion (sulfate or chloride) with the nose open and one for each anion with the nose occluded. There were at least 24 hours between sessions. The testing order of compounds and nasal conditions was counterbalanced over subjects. A single test session was conducted with both nasal conditions for sodium chloride. Sessions were conducted in the sensory evaluation facility in the Department of Food Science, Cornell University. Subjects were requested not to eat or drink anything for one hour preceding each session. Each session started with subjects rinsing their mouths three times with deionized water. One rinse of deionized water was also taken between stimuli. In the nasal occlusion condition, subjects wore Spirometrics Spiro Nose clips (Spiro No. 2110; Spirometrics Medical Equipment, Grey, ME). Tests were conducted under red light to mask any visual cues.

Detection thresholds were determined using a modified forced-choice ascending method of limits [17 - 20]. At each concentration, a three-sample set consisting of one test and two blank (deionized water) samples was presented to subjects. Positions of the target sample and blanks were counterbalanced over sessions and subjects. The subject was presented with the lowest concentration step and asked to indicate which of the three samples was different from the other two. After a 30 second break, subjects were presented with another set of three samples at a concentration 2.5 times higher. Judgments were completed when the subject either finished all concentrations or made three correct discriminations in a row. The best-estimate threshold for each subject was the geometric mean of the concentration at which the last incorrect choice occurred and the next higher concentration. Point estimations of threshold were also made based on the group percent correct at each concentration level. The chance-adjusted 50% point (66.6% correct) was found by interpolation from the linear segment of the function for percent correct plotted against log concentration, and from the logistic regression where the equation $\ln(p/1-p) = b_o + b_1 (\log C)$ was fit (p is proportion correct, C is concentration, and b's are slope and intercept constants).

3. Results

The best estimate (geometric mean), logistic regression and percent correct group detection thresholds of the iron and copper compounds are shown in Table 2. Analysis of the log transformed detection threshold concentrations was done for iron and copper salts separately using PROC MIXED SAS v.9.1 (SAS Institute Inc., Cary, NC), with nose condition and anion as factors and panelists as a random effect. Nasal occlusion raised thresholds for the metal salts but not for sodium chloride. There was a significant effect of nasal occlusion for both iron [$F(1,19) = 8.28$, $p = 0.01$] and copper [$F(1,19) = 4.76$, $p = 0.042$]. There were neither significant anion differences nor any nose condition by anion interactions. This coincides with other studies showing that the chemosensory effects of the anion are not as large as those of the cation [1, 8, 9, 21]. Sodium chloride thresholds were 1.53 mM (95% C.I. = 1.15 to 3.25) with the nose open and 1.93 mM (95% C.I. = 1.22 to 4.42) with the nose closed (no significant difference, $t(19) = .71$, $p = .49$). For NaCl, point estimates from interpolation at the chance-corrected percent correct were 4.81mM and 2.47 mM, for nose open and nose closed conditions, respectively.

The geometric means of the individual thresholds gave estimates that were lower than the values obtained using interpolation on a logistic regression and lower than values from interpolation on the percent correct function in eight of ten comparisons. However, results were similar in that all three methods showed the drop in metal salt thresholds with nasal occlusion, higher values for sodium and a similar rank order among compounds. Correlations among the 10 mean log thresholds from the three methods of analysis ranged from .82 to .94.

4. Discussion

Iron and copper compounds are often described as having a metallic taste or flavor [12,13, 22], which is considered undesirable in foods. Despite this metallic sensation, copper and iron are essential nutrients for humans and their salts are often used as fortifying agents [8, 23-25]. Threshold values obtained here for iron and copper salts are in a comparable range to previous findings but given the uncertainty in nasal conditions in most published studies and differences in methods, direct comparisons are difficult. The most direct comparison is to the data of Lim and Lawless [1] who used the same analysis method, procedure, water source, laboratory environment and subject pool. The current values are comparable to that study (nose open), while the values from the nose closed condition were closer to the higher values found by Schiffman [2] who also used nose clips. The increase in thresholds with nasal occlusion was significant for the iron and copper compounds but not for the sodium chloride control, as expected. Thus the increase is not likely due to any distraction or other effect of wearing nose clips per se, but rather the effect that they have on reducing retronasally perceived odors.

Nasal occlusion attenuates the perception of iron salts [12, 13] but the effect on copper in the literature is less clear [8, 12, 14]. The iron threshold values in this study are lower with the nose open than closed as shown by all three estimation methods in Table 2. This is consistent with the hypothesis that iron compounds give rise to a retronasal olfactory stimulus that is diminished by blockage of the nasal passage as suggested previously [1, 12-15, 26]. Copper compounds did not have as much of a retronasal component as iron, if this can be inferred from the magnitude of the difference in nasal conditions. In terms of the geometric mean values for ferrous sulfate and ferrous chloride there is a factor of 5.4 and 3.5 difference comparing the nose closed and nose open values. For copper sulfate and copper chloride there was a factor of 3.1 and 1.9 difference comparing the nose closed and nose open values. However, there may be a greater retronasal component arising from copper than previously thought.

Lim et al. [14] stated that solutions of ferrous sulfate had no smell when the headspace over the solutions was sniffed (orthonasal smell was absent), suggesting that the olfactory stimulus arises from rapidly generated lipid oxidation products in the mouth. If so, oral contact may be required for the retronasal sensation from iron to be developed. Further evidence for the contribution of lipid oxidation was shown by a recent study of skin contact with metals. Glindemann et al. [27] found the metallic odor from iron contacting the skin is a result of the skin converting the iron metal to form reactive Fe^{2+} ions that are oxidized to Fe^{3+} ions while simultaneously reducing skin lipid peroxides to carbonyl compounds that are perceived as metallic odor. Further research comparing ferrous compounds in the oral cavity with and without oral contact could examine whether oral contact is required for the production of the retronasal constituent. A lack of retronasal smell without oral contact would support the hypothesis that iron is catalyzing a lipid oxidation giving rise to metallic odorants. An oral isolation device such as the barrier used by Pierce et al. [28] could be used in such a comparison. Another potentially useful approach would be to analyze metal salt solutions mixed with saliva using gas chromatography effluent sniffing [29] , similar to the approach used by Glindemann et al. [27] to identify the compounds causing the metallic odor from skin contact.

The choice of analysis in the threshold estimation, i.e. how to estimate the threshold concentration, can affect the obtained values. The three alternative forced choice threshold method provides a rich data set with several possible analyses [17 - 19]. Three methods used here were the best estimate (geometric mean) from individual threshold estimates and interpolated values of the chance-adjusted 50% point from logistic regression and from inspection of simple group percent correct plots. Cuppett et al. [6] found a two-fold difference between threshold values calculated from geometric mean and logistic regression methods, but stated that this was not a substantial difference. In this study, the geometric means of individual (“best-estimate”) thresholds were lower than the interpolated values from the group percents correct in 8 of 10 comparisons (including the NaCl control), but correlations among the mean values were high. Interpolating a threshold point from a psychometric function also leaves some additional decisions open to the experimenter. How many points should be considered in fitting a line to the data? Robinson et al. [18] chose to use only the two points bracketing the chance corrected threshold level (75% in their R-index method). We chose to inspect each individual response plot to use a linear portion bracketing the chance-corrected level. These choices are additional methodological details to consider if one compares threshold values across studies that used different estimation methods.

In conclusion, iron and copper compounds exhibit increased thresholds with nasal occlusion, implying significant retronasal odor contributions to the overall sensation. The olfactory sensation often called a metallic “taste” at suprathreshold levels is also induced by an exposure to metal salts at low concentrations.

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Table 1

Summary of Previous Findings, Iron and Copper Thresholds

Primary Researcher	Test Method	Nose Condition	Sample Size (ml)	Compound(s)	Threshold Value (μM)	Water Type	Analysis
Cohen 1960	modified triangle test and duo-trio test	not stated	50	$FeSO_4$ CuCl+	12.2 6.5 38.7 74.5	distilled spring distilled spring	50% 50% 50% 50%
Zacharaias R. 1979	paired difference	not stated	25	$FeSO_4$	10.8	not stated	50%
Beguin-Bruhin 1983	5-AFC	not stated	30	$CuSO_4$	9.6-12.8 3.2-4.0	distilled mineral spring	Probit Probit
Gonzalez 1998	3-AFC	not stated	50	$FeSO_4$	28.4	mineral	GM
Schiffman 2000	3-AFC	closed	2	$FeSO_4$ $FeCl_2$	143.0 875.0	deionized deionized	MD MD
Zacarias I. 2001	modified triangle test	open	20	$CuSO_4$ $CuCl_2$	9.6 14.0 14.7 22.3	distilled deionized uncarbonated mineral distilled deionized uncarbonated mineral	50% 50% 50% 50%
Lim 2006	modified triangle test	open	20	$FeSO_4$ $FeCl_2$	99.2 66.0	deionized deionized	GM GM
Cuppett 2006	5-AFC	not stated	20	$CuSO_4$	0.48 0.39 0.77 0.75	distilled deionized mineral distilled deionized mineral	GM GM L L

AFC: Ascending forced choice

50%: Concentration when 50% of panelists detected compound

GM: Geometric Mean

L: Interpolated from logistic regression

MD: Mean Detection

Table 2

Threshold Values, μM

Compound	Geometric Mean (95% C.I.)		Percent Correct		Logistic Regression	
	Nose Open	Nose Closed	Nose Open	Nose Closed	Nose Open	Nose Closed
Ferrous Sulfate	29.9 (7.5, 120)	161 (60.1, 429)	9.5	313	8.2	234
Ferrous Chloride	64.0 (21.2, 193)	227(99.8, 515)	40.1	443	35.1	310
Copper Sulfate	7.8 (3.2, 19.0)	24.6 (11.1, 54.4)	22.1	102	18.9	592
Copper Chloride	8.2 (3.4, 19.5)	15.6 (6.5, 37.5)	27.3	48.4	28.4	39.5