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RESEARCH HIGHLIGHT

Nicotine and the taste allure for salty food

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Smoking has been recognized as one agent that may decrease the effectiveness of the gustatory system to detect salt (Na^+) in foodstuffs. As a consequence, smokers tend to ingest saltier foods than nonsmokers. An increase in sodium intake has been associated with hypertension: thus, smoking may concur to the development of hypertension by impairing salt perception. Understanding the mechanisms underlying the action of smoking on salty taste represents the premise to design proper intervention aiming at restoring normal sensitivity to sodium in smokers. I addressed this issue by studying the effect of nicotine, one of the main components of tobacco smoke, on the sodium detection mechanism in rat taste cells. Electrophysiological analysis of these cells revealed that long-term exposure to nicotine reduced the ion current mediated by the Epithelial Sodium Channel (ENaC), one of the sodium receptors occurring in taste cells. As to the molecular mechanism responsible for such a current decrease, data were consistent with a reduction in the number of functional ENaCs in the membrane of taste cells. Therefore, nicotine reduces the capability of taste cells to respond to sodium ions. This might explain, at least in part, why smokers tend to use salt more abundantly when flavoring their food: they are just boosting the sensory information to be relayed to the brain.

Keywords: taste cell; ENaC; patch-clamp recording; salt transduction; smoking

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Salt taste perception and smoking

Taste reception is a fundamental sensory activity for proper selection of food and beverages [1]. Among the several chemicals recognized by the taste system, sodium ions (Na^+) are of particular relevance. Na^+ represents the main cation of extracellular fluid and is a key factor in essential physiological processes undergoing in our body, from the generation and conduction of nerve impulses to the regulation of cell tonicity. Taste helps us to detect this mineral in foodstuffs: Na^+ elicits a specific sensation called salty taste that guides the ingestion of this cation (sodium intake) [2, 3].

Man evolved in low-sodium habitat and is well adapted to handle low salt availability. In modern life, however, salt (NaCl) is easily accessible and this has led to consumption beyond the physiological needs. Na^+ affects the extracellular fluid volume and therefore blood pressure: indeed, increase dietary sodium intake has been characterized as an important contributor to hypertension [4-6]. Hypertension is a major risk factor for many cardiovascular diseases including stroke, coronary heart disease, cardiac failure, and endstage renal disease. Recommendations to prevent the development of hypertension include a reduced sodium intake and avoiding

those factors, such as smoking, that may interfere with salt perception by taste system.

Smoking affects salt taste perception by raising the threshold for recognition and by reducing the perceived intensity at supra-threshold concentrations [7-12]. This means that smokers would consume more salt because they cannot taste it as well as nonsmokers, all things being equal. Indeed, smokers tend to ingest saltier foods than nonsmokers [13-17], and this expose them to the risk of developing hypertension. The actual amount of sodium ions ingested by smokers can be higher than 4 g (200 mmol) a day [17], that is, twice as much as the amount of sodium in a medium sodium diet (~100 mmol) [18]. An increase in sodium intake from ~1.5 g to ~3 g a day has been shown to induce an increase in the systolic blood pressure of ~8 mmHg [18, 19]. Thus, smoking may concur to aggravate hypertension by acting on salt perception, that is, by reducing the effectiveness of the taste system to detect salt.

Nicotine action on sodium receptors in rat taste cells

The mechanisms underlying the action of smoking on salt taste perception are currently unknown. I have addressed this issue in my recent work [20] by testing the following hypothesis: nicotine acts at the level of taste cells by disrupting the sensory mechanism involved in sodium detection. I put forward this working hypothesis on the basis of the following considerations.

Nicotine, one of the main components of tobacco smoke, is a pharmacologically active alkaloid that affects several tissues [21], including the peripheral sensory organs of taste, the taste buds [22-26]. Taste buds are made of taste cells, which detect chemicals dissolved in the saliva (including Na^+), transform them into electric impulses, and then relay sensory information to the brain [3, 27]. I therefore reasoned that impairment (worsening) of salt taste perception induced by smoking could be explained, at least in part, in terms of nicotine affecting the mechanism involved in the detection of Na^+ in taste cells.

Increases in the sodium concentration in the oral cavity are thought to be transduced through a direct entry of Na^+ into taste cells via open channels located in their apical membrane bathed by saliva [27, 28]. Among these, the Epithelial Na Channel (ENaC) represents one of the best characterized "sodium receptor" occurring in mammalian taste cells [29-31]. Thus, I hypothesized that nicotine could disrupt the functioning of this channel.

Active ENaCs can be monitored in single cell by analyzing the so-called response to amiloride with the electrophysiological technique of patch-clamp recording [32]. Amiloride is a diuretic drug that blocks ENaC in the sub-micromolar concentration range [27]. If a cell express functional ENaCs, application of amiloride to that cell causes a reduction in the ion current flowing through the membrane by shutting down these channels: this current reduction is referred to as the response to amiloride. I therefore tested whether nicotine affected the response to amiloride in taste cells of the rat, an animal model widely used in taste research.

To reproduce long term exposure to nicotine as found in smokers, drug was administered to rats via drinking water [25], allowing a continuous exposure [33] that resembles the one observed in habitual smokers [34, 35]. The oral administration route is also similar to the "chewing tobacco" or the "nicotine gum" route of exposure in humans [10, 36, 37].

The results of my research indicated that nicotine treatment exerted a clear-cut effect on taste cells by causing a significant decrease in the response to amiloride. This reduction could be explained in terms of different mechanisms. A reduced sensitivity to amiloride (expressed by the inhibition constant, K_i) would result in a reduced amplitude of the response to a given amiloride concentration. Similarly, a change in the ion selectivity (P_{Na}/P_K) of ENaCs in favor of potassium ions would cause a reduction in the response amplitude at any membrane potential. However, I found that both K_i and P_{Na}/P_K did not change after nicotine treatment.

It is well established that sensitivity of ENaC to amiloride inhibition depends on the subunit composition of the channel (which consists of three different subunits: α, β, and γ [38]). For example, variations in the expression level of the β or γ subunit would have a profound impact on K_i [39]. Thus, my electrophysiological results indicated that chronic exposure to nicotine likely did not affect the subunit composition of ENaCs in taste cells.

Covalent modifications of the channel protein (such as phosphorylation by protein kinase A or ADP ribosylation) may induce changes in ENaC ion selectivity [40, 41]. My results suggested that long-term exposure to nicotine unlikely induced covalent modification affecting P_{Na}/P_K ratio.

Assuming that the single-channel conductance of ENaC did not vary under nicotine treatment (as indicated by unchanged values for K_i and P_{Na}/P_K) [39, 40], then data could be interpreted on the basis of a reduction in the "activity" of the channels. Channel activity is defined as the number of functional channels (N_{ENaC}) times the channel open probability (P_O) [42]. Of course, a decrease in channel activity could be due to a

reduction in N_{ENaC} and/or to a decrease in P_O. Nonetheless, a reduced activity of functional ENaCs would produce a smaller electrical signal when taste cells are stimulated by increasing the external sodium concentration. Therefore, a reduced activity of ENaCs would be reflected in taste cells being less sensitive to sodium variations in the saliva.

How nicotine affects the activity of ENaCs remains to be elucidated. However, some clues for the possible mechanism have been provided by other electrophysiological observations. I found that that membrane capacitance (C_m) was about 25% larger in nicotine-treated cells than in control ones. This effect was specific, because C_m did not change in taste cells lacking ENaCs. C_m is directly related to the actual extension of the membrane lipid bilayer, and therefore C_m changes could reflect heavy membrane remodeling. Variation in the rate of endocytosis and exocytosis may have a dramatic effect on cell membrane extension. ENaC is subjected to a continuous turnover in other cell types. Typically, the total cellular pool of ENaC turns over rapidly, with a half-life of about 40-120 min in cultured cells [43]. The density of functional ENaCs on the cell membrane is therefore the result of the equilibrium between channel exocytosis and endocytosis [43]. Nicotine induces endocytosis of ENaCs in human nasal epithelium cells in culture with concomitant reduction in membrane capacitance [44]. Thus my findings suggest that nicotine could interfere with regular ENaC membrane trafficking in taste cells, although at the moment it is difficult to reconcile an increase in ENaC endocytosis (which would explain the reduction in the response to amiloride) with an increase in membrane surface area of taste cells (increased C_m).

Restoring sodium taste sensitivity in smokers?

Findings on rat taste cells indicate that chronic administration of nicotine inhibits the activity of ENaC, which represents the "sodium receptor" [30]. Therefore, nicotine impairs the first molecular step in sodium detection. The biological consequence of this effect would be a reduced inflow of sodium ions into taste cells when sodium concentration increases in the saliva (as it occurs during chewing of salty food). Thus, taste cells would produce smaller biological signals in response to salt stimulation and this would be translated in reduced sensory input to nerve fibers. In short, the reduced capability of taste cells to detect sodium ions would impair the sensory analysis performed by taste system on salty food.

It is conceivable that a similar mechanism could explain, at least in part, the reduced taste sensitivity to sodium ions (salty taste) in smokers. Both amiloride-sensitive (ENaC-mediated) and amiloride-insenstive components have been described for sodium taste in humans [45-53], although a great variability exists among individuals [52]. ENaC α, β and γ subunits occur in human fungiform papillae [54, 55]. On the basis of these evidences, it is tempting to speculate that the effect of nicotine on ENaC observed in rat taste cells could also take place in those individuals expressing the amiloride-sensitive sodium pathway. As a consequence, smokers would tend to add more salt in foods to compensate for a sodium detection mechanism partially disrupted by nicotine.

In conditions of reduced number of functional ENaCs, a simple intervention to restore (increase) sodium sensitivity of taste cells exposed to nicotine would be to enhance the activity of residual ENaCs. Several substances are known to act as positive modulators for these channels. For example, the amino acids arginine and lysine enhance currents through human ENaC expressed in *Xenopus* oocytes and enhance salt taste perception in human subjects [55]. Therefore, adding a positive modulator of ENaCs to table salt could be a possible remedy to correct the sensory deficit in salt perception caused by nicotine.

Conflicting interests

The author has declared that no conflict of interests exist.

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Abbreviations

C_m: membrane capacitance; ENaC: epithelial sodium channel; K_i: inhibition constant; N_{ENaC}: number of functional ENaCs; P_K: permeability to K^+; P_{Na}: permeability to Na^+; P_O: single-channel open probability.

Author contributions

A.B. conceived and conducted the experiments, analyzed the results, and wrote this manuscript.

References

1. Breslin PA. An evolutionary perspective on food and human taste. Curr Biol 2013; 23:R409-R418.
2. Mattes RD. The taste for salt in humans. Am J Clin Nutr 1997; 65(suppl):692S-697S.
3. McCaughey SA, Scott TR. The taste of sodium. Neurosci Biobehav Rev 1998; 22:663-676.

4. Batuman V. Salt and hypertension: why is there still a debate? Kidney Int Suppl 2013; 3:316-320.
5. Delahaye F. Should we eat less salt? Arch Cardiovasc Dis 2013; 106:324-332.
6. Ha SK. Dietary salt intake and hypertension. Electrolyte Blood Press 2014; 12:7-18.
7. Jackson JA. Heavy smoking and sodium chloride hypogeusia. J Dent Res 1967; 46:742-744.
8. Nilsson B. Taste acuity of the human palate. III. Studies with taste solutions on subjects in different age groups. Acta Odontol Scand 1979; 37:235-252.
9. Baker KA, Didcock EA, Kemm JR, Patrick JM. Effect of age, sex and illness on salt taste detection thresholds. Age Ageing 1983; 12:159-165.
10. Mela DJ. Gustatory function and dietary habits in users and nonusers of smokeless tobacco. Am J Clin Nutr 1989; 49:482-489.
11. Raffaelli R, Baldinetti A, Somma F, Rumi G, Tiberi F. Variations in the taste function of smokers. Minerva Stomatol 1989; 38:1253-1256.
12. Sato K, Endo S, Tomita H. Sensitivity of three loci on the tongue and soft palate to four basic tastes in smokers and non-smokers. Acta Otolaryngol Suppl 2002; 546:74-82.
13. Tell GS, Klepp KI, Vellar OD, McAlister A. Preventing the onset of cigarette smoking in Norwegian adolescents: the Oslo youth study. Prev Med 1984; 13:256-275.
14. Woodward M, Bolton-Smith C, Tunstall-Pedoe H. Deficient health knowledge, diet, and other lifestyles in smokers: is a multifactorial approach required? Prev Med 1994; 23:354-361.
15. Nishino Y, Fukao A, Tsubono Y, Tsuji I, Kuwahara A, Hisamichi S. Relation between passive smoking at home and dietary intake. Nippon Koshu Eisei Zasshi 1998; 45:619-624.
16. Kitagawa Y, Nakaji S, Shimoyama T, Umeda T, Sugawara K, Liu Q, *et al*. Differences in lifestyle of a smoking and non-smoking population in Japan. Asian Pac J Cancer Prev 2000; 1:245-249.
17. Mediavilla Garcia JD, Fernandez Torres C, Aliaga Martinez L, Leon Ruiz L, Sabio Sanchez M, Jimenez Alonso J. Clinical characteristics of patients with essential hypertension regarding salt intake. Rev Clin Esp 2001; 201:627-631.
18. Obarzanek E, Proschan MA, Vollmer WM, Moore TJ, Sacks FM, Appel LJ, *et al*. Individual blood pressure responses to changes in salt intake. Results from the DASH-sodium trial. Hypertension 2003; 42:459-467.
19. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, *et al*. Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (DASH) diet. N Engl J Med 2001; 344:3-10.
20. Bigiani A. Amiloride-sensitive sodium currents in fungiform taste cells of rats chronically exposed to nicotine. Neuroscience 2015; 284:180-191.
21. Yildiz D. Nicotine, its metabolism and an overview of its biological effects. Toxicon 2004; 43:619-632.
22. Dahl A, Erickson RP, Simon SA. Neural responses to bitter compounds in rats. Brain Res 1997; 756:22-34.
23. Ming D, Ruiz-Avila L, Margolskee RF. Characterization and solubilization of bitter-responsive receptors that couple to gustducin. Proc Natl Acad Sci USA 1998; 95:8933-8938.
24. Ming D, Ninomiya Y, Margolskee RF. Blocking taste receptor activation of gustducin inhibits gustatory responses to bitter compounds. Proc Natl Acad Sci USA 1999; 96:9903-9908.
25. Tomassini S, Cuoghi V, Catalani E, Casini G, Bigiani A. Long-term effects of nicotine on rat fungiform taste buds. Neuroscience 2007; 147:803-810.
26. Oliveira-Maia AJ, Stapleton-Kotloski JR, Lyall V, Phan TH, Mummalaneni S, Melone P, *et al*. Nicotine activates TRPM5-dependent and independent taste pathways. Proc Natl Acad Sci USA 2009; 106:1596-1601.
27. Lindemann B. Taste reception. Physiol Rev 1996; 76:719-766.
28. Stewart RE, DeSimone JA, Hill DL. New perspectives in gustatory physiology: transduction, development, and plasticity. Am J Physiol 1997; 272:C1-C26.
29. DeSimone JA, Lyall V. Taste receptors in the gastrointestinal tract. III. Salty and sour taste: sensing of sodium and protons by the tongue. Am J Physiol Gastrointest Liver Physiol 2006; 291:G1005-G1010.
30. Chandrashekar J, Kuhn C, Oka Y, Yarmolinsky DA, Hummler E, Ryba NJ, *et al*. The cells and peripheral representation of sodium taste in mice. Nature 2010; 464:297-301.
31. Roper SD. The taste of table salt. Pflügers Arch 2015; 467:457-463.
32. Bigiani A, Cuoghi V. Localization of amiloride-sensitive sodium current and voltage-gated calcium currents in rat fungiform taste cells. J Neurophysiol 2007; 98:2483-2487.
33. Liu J-J, Mohila CA, Gong Y, Govindarajan N, Onn S-P. Chronic nicotine exposure during adolescence differentially influences calcium-binding proteins in rat anterior cingulated cortex. Eur J Neurosci 2005; 22:2462-2474.
34. Benowitz NL, Florence K, Jacob P III. Circadian blood nicotine concentrations during cigarette smoking. Clin Pharmacol Ther 1982; 32:758-764.
35. Benowitz NL, Hansson A, Jacob P III. Cardiovascular effects of nasal and transdermal nicotine and cigarette smoking. Hypertension 2002; 39:1107-1112.
36. Parrott AC, Winder G. Nicotine chewing gum (2 mg, 4 mg) and cigarette smoking: comparative effects upon vigilance and heart rate. Psychopharmacology 1989; 97:257-261.
37. Benowitz NL. Systemic absorption and effects of nicotine from smokeless tobacco. Adv Dent Res 1997; 11:336-340.
38. Benos DJ, Stanton BA. Functional domain within the degenerin/epithelial sodium channel (Deg/ENaC) superfamily of ion channels. J Physiol 1999; 520:631-644.
39. McNicholas CM, Canessa CM. Diversity of channels generated by different combinations of epithelial sodium channel subunits. J Gen Physiol 1997; 109:681-692.
40. Ismailov II, Berdiev BK, Benos DJ. Biochemical status of renal epithelial Na+ channels determines apparent channel conductance, ion selectivity, and amiloride sensitivity. Biophys J 1995; 69:1789-1800.
41. Benos DJ, Fuller CM, Shlyonsky VG, Berdiev BK, Ismailov I.

Amiloride-sensitive Na+ channels: insights and outlooks. News Physiol Sci 1997; 12:55-61.

42. Marunaka Y, Eaton DC. Effects of vasopressin and cAMP on single amiloride-blockable Na channels. Am J Physiol 1991; 260:C1071-C1084.
43. Rotin D, Kanelis V, Schild L. Trafficking and cell surface stability of ENaC. Am J Physiol Renal Physiol 2001; 281:F391-F399.
44. Klimek T, Glanz H, Rückes-Nilges C, Van Driessche W, Weber WM. Nicotine-induced endocytosis of amiloride-sensitive sodium channels in human nasal epithelium. Acta Otolaryngol 2000; 120:286-290.
45. Schiffman SS, Lockhead E, Maes FW. Amiloride reduces the taste intensity of Na+ and Li+ salts and sweeteners. Proc Natl Acad Sci USA 1983; 80:6136-6140.
46. Tennissen AM. Amiloride reduces intensity responses of human fungiform papillae. Physiol Behav 1992; 51:1061-1068.
47. Ossebaard CA, Smith DV. Effect of amiloride on the taste of NaCl, Na-gluconate and KCl in humans: implications for Na+ receptor mechanisms. Chem Senses 1995; 20:37-46.
48. Smith DV, Ossebaard CA. Amiloride suppression of the taste intensity of sodium chloride: evidence from direct magnitude scaling. Physiol Behav 1995; 57:773-777.
49. Tennissen AM, McCutcheon NB. Anterior tongue stimulation with amiloride suppresses NaCl saltiness, but not citric acid sourness in humans. Chem Senses 1996; 21:113-120.
50. Anand KK, Zuniga JR. Effect of amiloride on suprathreshold NaCl, LiCl, and KCl salt taste in humans. Physiol Behav 1997; 62:925-929.
51. Ossebaard CA, Polet IA, Smith DV. Amiloride effects on taste quality: comparison of single and multiple response category procedures. Chem Senses 1997; 22:267-275.
52. Feldman GM, Mogyorósi A, Heck GL, DeSimone JA, Santos CR, Clary RA, *et al*. Salt-evoked lingual surface potential in humans. J Neurophysiol 2003; 90:2060-2064.
53. Feldman GM, Heck GL, Smith NL. Human salt taste and the lingual surface potential correlate. Chem Senses 2009; 34:373-382.
54. Rossier O, Cao J, Huque T, Spielman AI, Feldman RS, Medrano JF, *et al*. Analysis of a human fungiform papillae cDNA library and identification of taste-related genes. Chem Senses 2004; 29:13-23.
55. Stähler F, Riedel K, Demgensky S, Neumann K, Dunkel A, Täubert A, *et al*. A role of the epithelial sodium channel in human salt taste transduction? Chem Percept 2008; 1:78-90.