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The soothing effect of menthol, eucalyptol and high-intensity cooling agents

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ABSTRACT

Several compounds are commonly used as flavours in foods such as chewing gum and candy to generate a pleasant cool sensation which can have a soothing effect on the upper respiratory tract. Menthol and eucalyptol are the flavours most widely used as cooling agents and their effect is supported by scientific data. The cool sensory perception they induce is due to their ability to activate cold receptors, particularly the transient receptor potential cation channel subfamily M member 8 (TRPM8), found in the skin and oral cavity. The ability to activate cold receptors has been also shown for non-volatile and non-menthol coolants developed since the 1970s, assessed as Generally Recognized as Safe and approved for use in food by the Flavor and Extract Manufacturers Association of the United States. Among these coolants, ethyl 3-(p-menthane-3-carboxamido)acetate, its analogue N-[[5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]glycine propyl ester and N-ethyl-p-menthane-3-carboxamide have demonstrated a greater cooling effect than menthol and are defined as 'high-intensity cooling agents'. Studies in human volunteers who consumed flavoured chewing gum found that the flavour intensity of menthol or eucalyptol, and consequently their cooling effect, increases with chewing and that volatile flavour compounds in the oral cavity are forced into air exhaled through the nasal compartment. It was thus confirmed that the soothing effect and increased air flow perceived in the nose and throat are sensations and not pharmacological actions.

Keywords

Flavors
Menthol
Eucalyptol
High-intensity cooling agents
Transient receptor potential cation channel

Menthol and eucalyptol: two flavouring agents for a pleasant sensation

Various compounds are commonly used as flavours in different products, such as chewing gum and candy, to generate a pleasant cool sensation which can have a soothing effect on the upper respiratory tract. Menthol and eucalyptol are the flavours most widely used as cooling agents and their effect has been scientifically proven. Menthol and eucalyptol are added as ingredients to a variety of consumer products, including pharmaceuticals, pesticides, cosmetics, liqueurs, toothpaste, shampoo and soap for their cooling and/or flavour-enhancing effects [**1, 2**].

Menthol is a naturally occurring compound of plant origin which gives plants of the *Mentha* genus their typical minty smell and flavour. It is a cyclic terpene alcohol with three stereogenic carbon atoms in its cyclohexane ring and thus occurs as four pairs of optical isomers, namely (+)- and (–)-isomenthol, (+)- and (–)-menthol, (+)- and (–)-neomenthol, and (+)- and (–)-neoisomenthol (Fig. 1).

The main form of menthol found in nature is (–)-menthol (also called L-menthol) which is the form most commonly used because it has greater cooling effects than the other isomers [**2**].

Menthol is chiefly obtained naturally from *Mentha canadensis* with the world production of L-menthol in 2007 estimated at about 19,000 tonnes. In addition, about 6,300 tonnes are chemically synthesized annually through stereospecific synthesis from limonene, citronellal, cresol or other precursors. Menthol-like cooling compounds with a stronger cooling action than menthol but lacking the typical minty smell have been developed and are widely used in commercial products, such as candy and chewing gum [**3**].

Eucalyptol is also known as cineole or 1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane (Fig. 1). It is found in high concentrations in tea tree, mugwort, bay leaves and cannabis, and is the primary terpene (up to 80% by weight) in eucalyptus oil.

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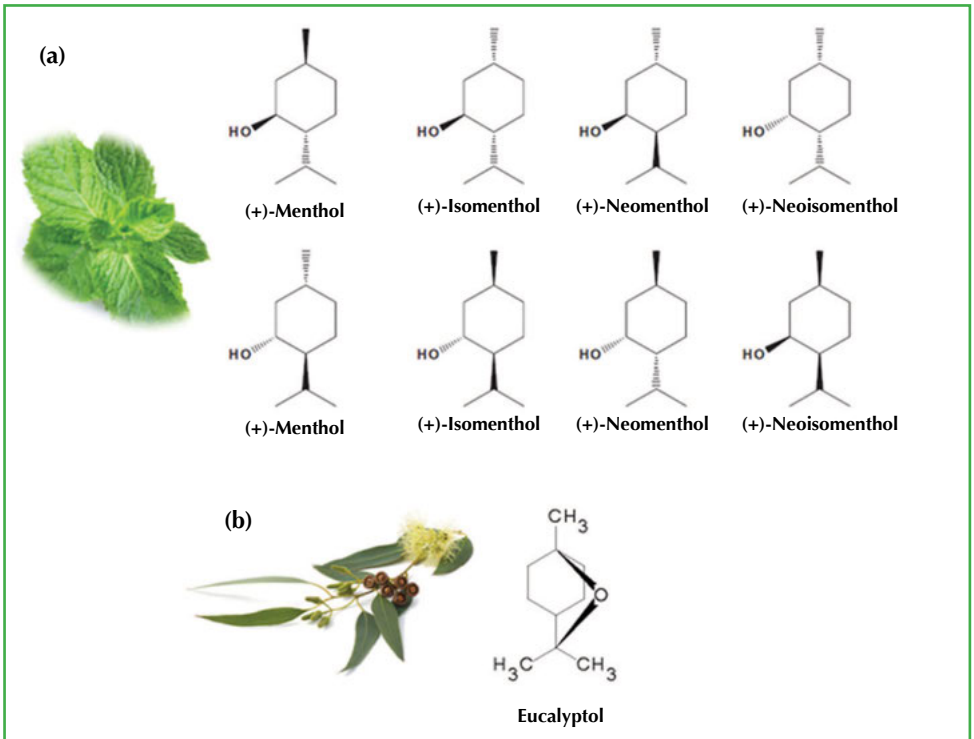

Figure 1 - Chemical formulae of (**a**) menthol and its stereoisomers and (**b**) eucalyptol

Menthol, eucalyptus oil and eucalyptol for therapeutic use were obtained from natural sources before compounds with controlled and demonstrated pharmacological activity were developed.

The cooling sensation elicited by menthol and eucalyptol

When products containing menthol or eucalyptol are inhaled, chewed, consumed or applied to the skin, they produce a cooling effect or sensation. This mechanism has been widely investigated. During the last 20 years, numerous studies have also examined the relationship between food consumption and retronasal aroma perception. The timing and physiological activity of the organs involved in mastication, deglutition and swallowing play a major role in this process. The oral cavity is connected to the airways, allowing free passage of odorants through the nasal cavity via the retronasal route. During chewing, the mouth seems to act like a bellows, mixing flavoured air with exhaled air which then flows past the olfactory epithelium lining the nose. Experimental data suggest that both the orthonasal and retronasal pathways employ the same olfactory system. Aroma transport to the nose involves a series of alternating static and dynamic events, which are affected by the texture and amount of food in the oral activity and behaviour patterns while eating.

Early studies with menthol in healthy volunteers showed that menthol vapours did not act as a decongestant but did produce a sensation of increased air flow in the nose [**4, 5**], suggesting an interaction between menthol and specific receptors.

The cool sensations elicited by menthol and eucalyptus were attributed to stimulation of thermoreceptors and in fact, it was subsequently demonstrated that menthol and cold stimuli share a common site of action. The menthol receptor was then cloned and characterized using a cDNA expression library and genomic DNA database, and the 1,104 amino acid transient receptor potential cation channel subfamily M member 8 (TRPM8) was identified [**6, 7**] (Fig. 2). This channel could be activated by both menthol and thermal stimuli in the cool to cold range of 8–28°C, demonstrating that menthol elicits a cooling sensation by acting as an agonist to a thermally sensitive receptor [**1, 7**].

TRPM8 is a member of the transient receptor potential (TRP) family of excitatory ion channels [**7**]. Six temperature-sensitive TRPs found in the skin and oral cavity are sensitive

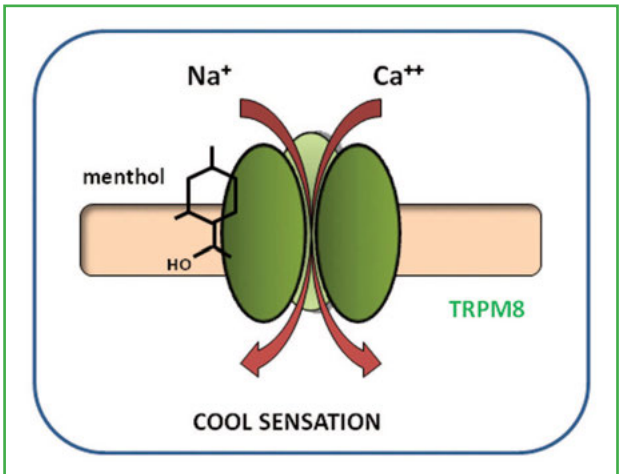

Figure 2 - Schematic representation of the TRPM8 receptor channel. When menthol binds to the receptor, sodium (Na^+) and calcium (Ca^{++}) ion fluxes are activated

to temperatures ranging from noxious heat to noxious cold, and include TRPV1, TRPV3, TRPM8 and TRPA1 [**8**]. TRPV1 is activated by capsaicin (the active component of chili peppers) and camphor (an aromatic botanical derived from the *Cinnamonum camphora* tree) as well as heat. TRPV3 is activated by heat and camphor. The TRPM8 and TRPA1 receptors, members of the melastatin and ankyrin repeat subfamilies, respectively, can both be activated by a range of cooling temperatures [**1**]. Natural compounds that evoke cooling sensations, such as menthol, eucalyptol and icilin (a synthetic compound unrelated to menthol) activate TRPM8, thus rapidly increasing intracellular calcium and mobilizing calcium flux through the channels [**1, 9**] (Fig. 3). This cooling effect can last 70 min or more in about 65% of human subjects [**10**]. Several other naturally occurring terpenes exert physiological cooling properties. Among them, only isopulegol and cis-p-menthane-3,8-diols showed a significant effect on TRPM8 channel activation.

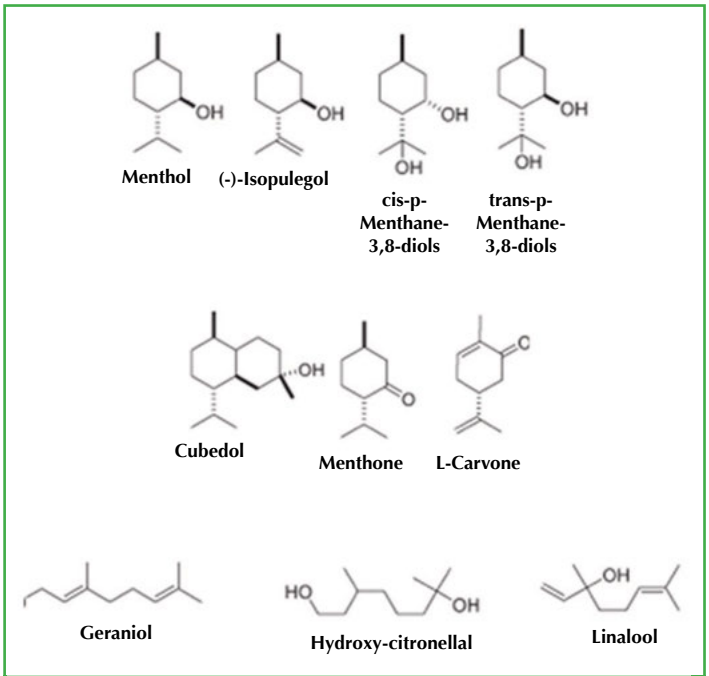

Figure 3 - Chemical formulae of commonly used cooling agents

There is little information on the effect of menthol concentration on perceived effect. Psychophysical studies have shown that menthol evokes a cool sensation in the skin or mucous membrane, but only at low concentrations, whereas high concentrations elicit a sensation of burning, irritation and pain, sometimes with local anaesthesia [**3**].

Haahr *et al*. [**11**] investigated whether oral function affected the release of peppermint-flavoured compounds from chewing gum. Ten healthy young male volunteers chewed peppermint chewing gum (960±2 mg) unilaterally on their preferred chewing side. The coated chewing gum pieces contained 2% flavour compounds (L-menthol and menthone), 58% sweetener (xylitol and sorbitol) and 40% gum base. Three different chewing intensities (habitual chewing frequency and frequencies of 60 and 88 chewing strokes/min) were considered and the levels of menthol and menthone in saliva and expired air from the nose were determined at different times after chewing. Menthol and menthone concentrations rose in saliva within the first 5 min, independently of their functional chemical group, and then passed along the retronasal route. Flavour intensity, and consequently its cooling effect, increased during chewing and swallowing. Concentrations were higher in the breath of volunteers chewing at 88 strokes/min than at the other chewing frequencies, indicating that aroma release and transfer is facilitated by the rate of mastication.

High-intensity cooling agents

Since the 1970s, the commercial need for non-volatile cooling agents lacking the negative effects of menthol (i.e., burning, irritation and pain at high concentrations) has stimulated flavour companies to develop new compounds. The use of non-menthol coolants in food applications increased in the 1990s with the approval of many cooling agents as Generally Recognized as Safe (GRAS). For example, N-ethyl-p-menthane-3-carboxamide (WS-3) was approved in 1975 for use in beverages, frozen desserts, confections, jams and chewing gums by the Flavor and Extract Manufacturers Association of the United States (FEMA) which assessed it as GRAS [12, 13]. WS-3 is used alone or in combination with menthol or other cooling compounds, like 2-isopropyl-N,2,3-trimethylbutyramide (WS-23), which was approved in 1996. Other coolants have since been synthesized, including Frescolat® MGA, Frescolat® ML and Coolact® 10 (3-L-menthoxypropane-1,2-diol) [**13, 14**].

The cooling intensity of 18 common coolants was recently evaluated in humans by Johnson *et al*. [**15**] using a 0–9 scale, with 0 being the least intense sensation and 9 the most intense (Fig. 4). A calibration test was first performed where each volunteer rinsed their mouth for 10 s with 10 ml of an aqueous solution containing 5% sucrose and 100 ppm L-menthol; this solution was assigned a cooling intensity of 5 (Fig. 4). After resting for 10 min during which they consumed unsalted crackers and water, each volunteer rinsed their

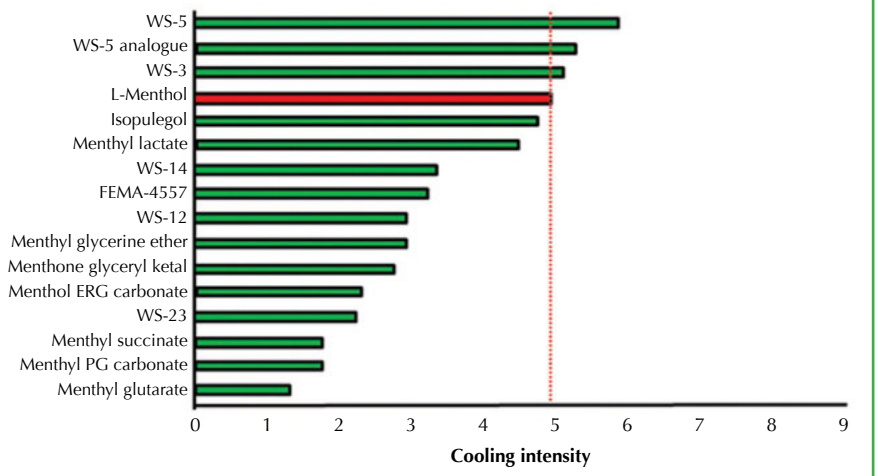

Figure 4 - Evaluation in human subjects of the cooling intensity of 18 cooling agents using a 0–9 scale. Cooling solutions contained 100 ppm coolant and 5% sucrose. An L-menthol solution was used for calibration. Adapted from Johnson *et al*. [**15**]

mouth for 10 s with 10 ml of an aqueous solution containing 5% sucrose and different coolants. The cooling sensation was rated against the solution containing 100 ppm menthol. Ethyl 3-(p-menthane-3-carboxamido)acetate (WS-5) and its analogue N-[[5-methyl-2-(1-methyllethyl)cyclohexyl]carbonyl]glycine propyl ester demonstrated the highest cooling intensity and, together with WS-3, demonstrated a cooling intensity greater than that of L-menthol [**15**] (Fig. 4). Isopulegol and methyl lactate showed a cooling intensity similar to that of L-menthol, with all other coolants demonstrating lower activity (Fig. 4). The cooling sensation results have been correlated with the ability of the compounds to activate the TRP channels, in particular TRPM8 and TRPA1 [**15**]. WS-5, which had the highest cooling intensity, was able to activate both the TRPA1 and TRPM8 receptor channels, the half maximal effective concentration (EC_{50}) values being 53.8 μM for TRPA1 and 0.5 μM for TRPM8. On the other hand, WS-3 primarily activated TRPM8 (EC_{50} values >100 μM for TRPA1 and 5.9 μM for TRPM8) indicating that the human perception of cooling intensity correlates well with the ability of the coolants to activate only one or both TRPM8 and TRPA1 receptors [**15**].

Conclusions

Reports in the literature indicate that menthol and eucalyptol, when inhaled, chewed in gum or consumed in other confectionery products, interact with specific receptors, generating transient sensations of cold, increased air flow and well-being. These sensations are collectively referred to as producing a 'soothing effect'. The ability to activate the TRP channels, in particular TRPM8 and TRPA1, has been also demonstrated for non-volatile cooling agents developed by flavour companies. Some of these agent exert a greater cooling effect than menthol and are defined as 'high-intensity cooling agents'.

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Human and Animal rights

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Informed consent

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Conflict of interest

The Department of Molecular Biochemistry and Pharmacology of the IRCCS-Istituto di Ricerche Farmacologiche "Mario Negri" has received fees for scientific consultancies from Perfetti Van Melle SpA within the last 3 years.

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