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The Oral Sensation of Carbonated Water: Cross-desensitization by Capsaicin and Potentiation by Amiloride

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Abstract

The oral sensation elicited by carbonated water is reduced by capsaicin and by blockers of carbonic anhydrase. We have investigated the temporal profile of this sensation and its cross-desensitization by capsaicin. We additionally tested if the sensation is influenced by amiloride. Following pretreatment of half of the dorsal tongue with 33 p.p.m. capsaicin, carbonated water was flowed over the tongue bilaterally for 5, 15 or 60 s. Subjects then performed a two-alternative forced choice test by indicating which side of the tongue had a stronger sensation and separately rated the sensory intensity on each side. Capsaicin significantly reduced the intensity of sensation elicited by carbonated water, consistent with cross-desensitization. This effect was weaker at 60 s because of a significant decline (desensitization) in ratings of the intensity of carbonated water on both sides of the tongue. Pretreatment with amiloride resulted in a small but significant increase in the intensity of the sensation elicited by the 15 s carbonated water stimulus, suggesting an amiloride-sensitive transduction mechanism.

Introduction

The tingling sensation elicited by carbonated water appears to be largely chemogenic. Acetazolamide, a blocker of carbonic anhydrase, depresses responses of sensory fibers in the lingual nerve and chorda tympani of rats elicited by carbonated water (Komai and Bryant, 1993; Komai *et al.*, 1994). Both acetazolamide (Dessirier *et al.*, 2000) and dorzolamide (Simons *et al.*, 1999) significantly attenuated the sensation evoked by carbonated water in humans, as well as the activity in rat trigeminal caudalis neurons that are involved in the transmission of oral irritation. These data indicate that the oral sensation from carbonated water is due to activation of chemosensitive nerve endings by carbonic acid, which is formed from carbon dioxide (CO_2) in a reaction catalyzed by the ubiquitous enzyme carbonic anhydrase (Wong *et al.*, 1983; Riley *et al.*, 1984; Neubauer, 1991; Christie *et al.*, 1995).

The oral sensation evoked by carbonated water is mediated partly by capsaicin-sensitive nociceptors. Capsaicin pretreatment resulted in a small but significant reduction in the intensity of the sensation elicited by carbonated water using a tongue-dip procedure (Dessirier *et al.*, 2000). We here wished to confirm this result using a higher capsaicin concentration and a better controlled method of application of carbonated water by flow. This procedure additionally allowed us to investigate the temporal profile of capsaicin cross-desensitization.

The exact site and cellular mechanism by which carbonic acid excites oral nociceptors is not known. Responses of cutaneous nociceptors to application of a solution saturated with CO_2 were facilitated by the sodium channel blocker amiloride; this effect was attributed to blockade of the Na^+/H^+ exchanger (NHE) (Steen *et al.*, 1999). The NHE is expressed ubiquitously (Krump *et al.*, 1997) and serves to regulate cell volume and intracellular pH (Lang *et al.*, 1998). Amiloride was also recently shown to enhance sustained inward currents elicited by acidic stimulation in trigeminal ganglion neurons (Liu and Simon, 2000). These findings predict that the oral sensation elicited by carbonated water should be enhanced by amiloride and we have tested this prediction in a final experiment.

Materials and methods

Subjects

A total of 55 healthy individuals (29 male, 26 female, aged 19–50 years) volunteered to participate in the experiments. All had refrained from consuming spicy food for 2 days prior to the experiment and had fasted for at least 1 h before testing, as verified by questionnaire. The study was

approved by the University of California–Davis Human Subjects Review Committee.

Stimulus application procedures

Carbonated water was prepared by pressurizing deionized water at room temperature with 95% CO_2 gas at 50 p.s.i. for 2 days. The pH of the resulting solution was 3.4 (± 0.1). To apply carbonated water subjects extended their tongue over a basin and carbonated water was delivered from the carbonator under CO_2 pressure at a rate of 20 ml/s onto the dorsal surface of the tongue. Carbonated water was delivered separately and simultaneously to each side of the tongue via a Y-shaped outlet.

Capsaicin (33 p.p.m.) was prepared by dilution of a 0.1% stock solution (3.3 mM in 95% ethanol) with dH_2O. Capsaicin was applied onto one side of the anterior dorsal tongue while dH_2O was applied onto the other side using cotton swabs (Puritan; Hardwood Product Co., Guilford, ME). The side of the tongue receiving capsaicin was counterbalanced across subjects. Two cotton swabs, one soaked in the capsaicin solution and the other with dH_2O, were simultaneously rolled along the dorsum of the tongue from the tip toward the back of the mouth and back in one stroke. The first stroke was made on each side of the midline, the second stroke approximately midway between the midline and the sides of the tongue and the last stroke close to the sides. A 20 min rest period was then imposed to allow capsaicin desensitization to develop.

Amiloride was dissolved in dH_2O to a concentration of 2 mM and was applied onto one side of the tongue while dH_2O was applied simultaneously onto the other side using cotton swabs as described above for capsaicin. The amiloride was applied in this manner twice with 1 min between applications, followed 1 min later by delivery of carbonated water.

Rating procedures

Carbonated water was applied by constant flow to both sides of the tongue for either 5, 15 or 60 s (see below). At the end of the stimulus period the subjects performed a two-alternative forced choice (2-AFC) task by indicating whether the capsaicin-pretreated or non-treated side of the tongue yielded a stronger sensation. They additionally rated the intensity of the sensation on each side separately using a category scale labeled 0 (no sensation) at one end and 10 (intense sensation) at the other end.

Experimental conditions

Capsaicin cross-desensitization to carbonated water (5 and 15 s)

After unilateral capsaicin pretreatment a group of 23 subjects (9 male, 14 female, aged 19–23 years) received carbonated water bilaterally for 5 s followed by the 2-AFC test and bilateral intensity ratings. After a 1 min rest period the carbonated water flow was resumed but this time for 15 s, with the 2-AFC task and intensity ratings obtained during the last 5 s of stimulation. To ensure that subjects rated only the last 5 s of the stimulus the investigator prompted them when the first 10 s of stimulation had elapsed. To avoid any potential desensitizing or sensitizing effect of the longer stimulus the 15 s stimulus was always administered after the shorter 5 s stimulus.

Capsaicin self-desensitization

At the end of the preceding experiment 15 of the 23 subjects were tested to verify capsaicin self-desensitization at the end of the session. For this, two 1 cm diameter filter paper disks soaked with 15 μl of the same 33 p.p.m. capsaicin solution were placed onto each side of the dorsal surface of the tongue. Thirty seconds later subjects performed the same 2-AFC and rating tests.

Capsaicin cross-desensitization to carbonated water (5, 15 and 60 s)

A separate group of 21 subjects (15 male, 6 female, aged 19–50 years) was tested with a carbonated water stimulus of 60 s duration. The reason for adding this group was that in the first experiment there was a trend toward increased intensity ratings on the treated side for the 15 s compared to the 5 s stimulus (see Results) and we wished to determine if this became significant with a more prolonged 60 s stimulus. The protocol described in experiment 1 above was followed, with the addition that 1 min after the 15 s stimulus carbonated water was applied again for 60 s, at which time subjects undertook the 2-AFC task and gave bilateral intensity ratings.

Amiloride

In a last group of 20 subjects (5 male, 15 female, aged 18–24 years, nine of whom had participated in earlier experiments with capsaicin) amiloride was applied unilaterally as described above, followed by bilateral application of carbonated water for 5 and 15 s. After each stimulus the 2-AFC test was undertaken and bilateral intensity ratings were obtained.

Statistical analysis

Data from the 2-AFC test were subjected to a binomial analysis of the proportion of correct choices, as well as d' analysis (Ennis, 1993; Bi *et al.*, 1997). While the binomial analysis merely assesses whether one side of the tongue was chosen in a significant majority of subjects, the d' analysis provides a measure of the difference between the two sides of the tongue that is independent of the discrimination method used and can be compared with results obtained in other studies. Significance of d' can be assessed using the method described (Bi *et al.*, 1997). Intensity ratings for each side of the tongue were compared by Student's *t*-test. For all significant differences reported $P < 0.05$.

Results

Capsaicin cross-desensitization to carbonated water

With the 5 s carbonated water stimulus a significant majority (37/44, $P < 0.001$, binomial) chose the non-pretreated side as having a stronger sensation (Figure 1B). This corresponded to a significant group d' value of 1.41 ($P < 0.001$). In addition, the mean intensity ratings on each side were significantly different ($P < 0.001$, t-test; Figure 1A). The higher d' value obtained in the present study (1.41) than in our prior study ($d' = 0.95$) might be attributed to the higher capsaicin concentration used and/or the method of stimulus delivery.

With the 15 s carbonated water stimulus the same proportion chose the untreated side as stronger (Figure 1B), and the difference in the mean intensity ratings between the two sides of the tongue was significant ($P < 0.001$, t-test; Figure 1A), although smaller compared with the 5 s stimulus.

With the 60 s carbonated water stimulus the difference in sensory intensity between the capsaicin-treated and untreated sides was much smaller. The proportion of subjects choosing the untreated side as stronger was no longer significant (13/21, $P = 0.38$, binomial; Figure 1B), corresponding to a non-significant d' value of 0.43 ($P = 0.14$). This was due mainly to a decrease in the carbonated water-induced sensation on the untreated side rather than to an increase on the capsaicin-pretreated side (Figure 1A), with the mean rating on the non-treated side significantly lower at 60 compared with 5 s ($P = 0.003$, t-test). The difference between the mean intensity ratings for the two sides of the tongue was, however, still significant ($P = 0.038$, t-test; Figure 1A).

On the capsaicin-treated side there were larger inter-individual differences in the pattern of sensory ratings for carbonated water stimuli of progressively longer duration, with an overall increase from 5 to 15 s and then a decline (Figure 1A). Thus, eight subjects (of 21 tested) gave higher ratings for the 5 s compared with the 60 s stimulus, while another eight did the reverse and the remainder reported similar intensities for both stimuli.

Capsaicin self-desensitization

With unilateral capsaicin each of the 15 subjects reported that the side that had not previously received capsaicin had a stronger irritation ($P < 0.001$, binomial) and significantly higher intensity ratings were assigned to that side (6.1 versus 3.8, $P < 0.001$, t-test). This confirms capsaicin self-desensitization as shown in earlier studies (Green, 1989; Dessirier *et al.*, 1997).

Amiloride

Amiloride pretreatment had no effect on the sensation elicited by the 5 s carbonated water stimulus, with a non-significant proportion of subjects (11/20, $P = 0.82$, binomial) choosing the untreated side as stronger (Figure 2B). The difference in mean intensity ratings between the two sides was not significant ($P = 0.4$, t-test; Figure 2A). However, amiloride pretreatment resulted in a significant increase in the intensity of sensation elicited by the 15 s carbonated water stimulus. A significant majority of subjects (15/20, $P = 0.041$, binomial) chose the amiloride-pretreated side as having a stronger sensation (Figure 2B), which corresponded to a significant group d' value of 0.95 ($P = 0.02$). Moreover, the mean intensity rating on the amiloride-pretreated side was significantly higher ($P = 0.008$, t-test; Figure 2A).

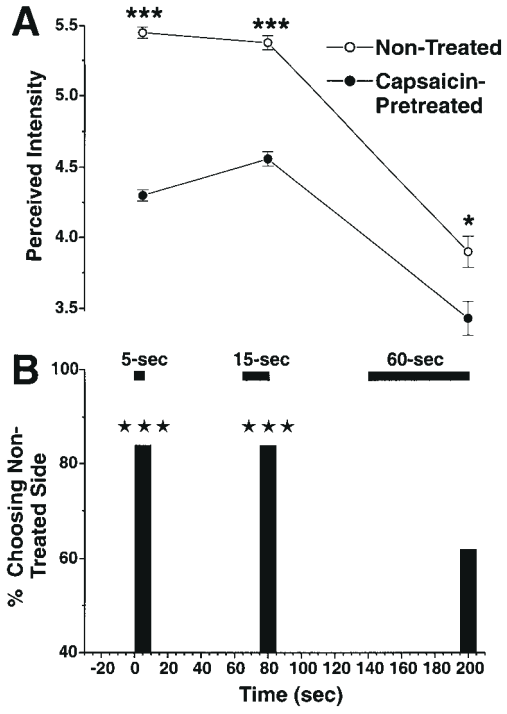

Figure 1 Capsaicin cross-desensitization of the sensation evoked by carbonated water. **(A)** Graphs of perceived intensity ratings on the capsaicin-pretreated and non-treated sides of the tongue for 5, 15 and 60 s carbonated water stimuli [same time axis as in (B)] given sequentially (durations of carbonated water stimuli indicated by filled horizontal bars). Error bars, SE; asterisks, significant difference between capsaicin-pretreated and non-treated sides (***$P < 0.001$, *$P < 0.05$, t-test). **(B)** Bar graphs of percentage of subjects that chose the non-treated side of the tongue as having stronger sensation in the 2-AFC test. ★★★, a significant majority chose the non-treated side ($P < 0.001$, binomial).

Discussion

Capsaicin was found to cross-desensitize the oral tingling sensation elicited by carbonated water. This confirms our earlier study using a different (tongue dip) stimulus procedure and lower (5 p.p.m.) capsaicin concentration (Dessirier *et al.*, 2000) and further implicates capsaicin-sensitive nociceptive afferent fibers in the mediation of this sensation. We also found that the sensation elicited

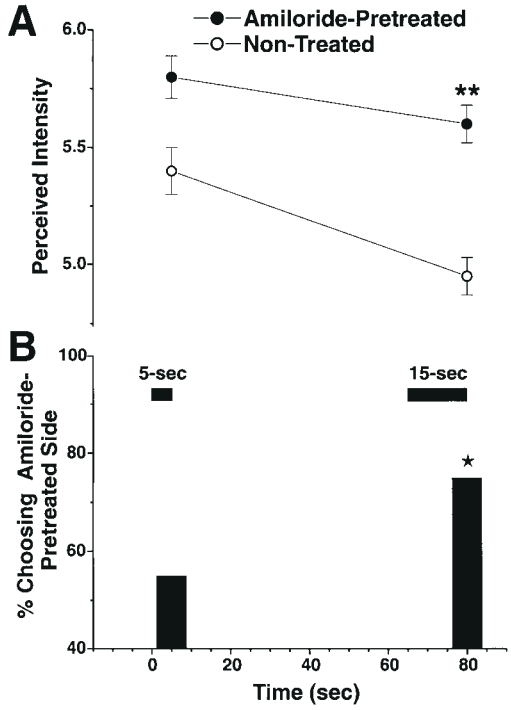

Figure 2 Amiloride enhancement of the sensation evoked by carbonated water. **(A)** Graphs of perceived intensity ratings on the amiloride-pretreated and non-treated sides of the tongue for 5 and 15 s carbonated water stimuli (format as in Figure 1). Carbonated water stimuli indicated by filled horizontal bars. **, significant difference between amiloride-pretreated and non-treated sides ($P < 0.01$, *t*-test). **(B)** Bar graphs of percentages of subjects choosing the amiloride-pretreated side as having a stronger sensation for the 5 and 15 s carbonated water stimuli. ★, significant majority chose amiloride-treated side ($P < 0.05$, binomial).

by carbonated water decreased during prolonged application, indicative of desensitization, similar to menthol and nicotine (Cliff and Green, 1994, 1996; Dessirier *et al.*, 1997, 1999). Finally, with amiloride there was a small but significant increase in the intensity of the perceived sensation of carbonated water. These findings are discussed in relation to possible physiological mechanisms underlying the sensation.

The sensation evoked by carbonated water decreased with stimulus duration (Figure 1), consistent with desensitization. There was considerable individual variability in ratings at 5 versus 15 s while the reduction in sensory intensity at 60 s was more consistent. The variability at 5 and 15 s might reflect competing sensitizing and desensitizing effects, as observed recently with menthol irritation (Dessirier *et al.*, 2001).

Mean intensity ratings were significantly lower on the capsaicin-treated side at all stimulus durations (Figure 1), indicative of cross-desensitization. Curiously, the mean rating on the capsaicin-treated side was higher at 15 compared with 5 s (Figure 1), possibly reflecting cross-stimulus-induced recovery from capsaicin desensitization (Green, 1996) which was then overcome by desensitization.

Following pretreatment with amiloride sensory intensity ratings were higher with both 5 and 15 s carbonated water stimuli, the latter being significantly higher (Figure 2B). The temporal profile of this effect has several possible explanations. First, the additional 60 s between the 5 versus 15 s carbonated water applications allowed more time for amiloride to act. Secondly, because sensory ratings were lower for the 15 versus 5 s stimulus (Figure 2), any incremental change in intensity would be more readily perceived from a lower rather than higher baseline level of intensity (Weber's Law effect). Third, amiloride may have reduced desensitization to the carbonated water, although this seems less likely since only eight of 20 subjects reported a decrease in sensory intensity on the non-treated side with the longer stimulus.

The present findings with amiloride are consistent with a previous electrophysiological study in which the responses of single cutaneous polymodal nociceptors to a saturated CO_2 solution were potentiated by amiloride (Steen *et al.*, 1999). This effect was postulated to be due to amiloride blockade of NHE. NHE is activated by hyperosmotic stimuli (i.e. cell shrinkage due to osmosis) or intracellular acidification to exchange intracellular protons for extracellular Na^+ ions in a 1:1 stoichiometry [for reviews see Madshus and Lang *et al.* (Madshus, 1988; Lang *et al.*, 1998)]. Because CO_2 in carbonated water is highly diffusable, it can easily penetrate the lingual epithelium and membranes of nociceptor endings where it could be enzymatically converted to carbonic acid intracellularly. Amiloride blockade of NHE would prevent nociceptor endings from counteracting the drop in intracellular pH, thus possibly contributing to an increased response.

Alternatively, the transduction mechanism for CO_2 might require its extracellular conversion to carbonic acid, where protons could bind to acid-sensitive ion channels of the amiloride-sensitive Na^+ channel/degenerin family that are expressed in the membranes of nociceptor endings [for recent reviews see Waldmann and Lazdunski (Waldmann and Lazdunski, 1998; Waldmann *et al.*, 1999)]. Acidic stimulation (pH 4.9) was recently shown to elicit a sustained inward current that was enhanced on average by 54% in the presence of 200 μM amiloride in some trigeminal ganglion neurons (Liu and Simon, 2000), consistent with our present findings.

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