

Ibuprofen as a Chemesthetic Stimulus: Evidence of a Novel Mechanism of Throat Irritation

Paul A.S. Breslin, Tara N. Gingrich and Barry G. Green^{1,2}

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, ¹The John B. Pierce Laboratory, 290 Congress Ave, New Haven, CT 06519 and ²Section of Otolaryngology, Yale University School of Medicine, New Haven, CT 06519, USA

Correspondence to be sent to: Paul A.S. Breslin, Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA.
e-mail: breslin@monell.org

Abstract

This paper reports a study of the oral and pharyngeal chemesthetic effects of the non-steroidal anti-inflammatory drug (NSAID) ibuprofen [2-(4-isobutylphenyl)propanoic acid], which pilot experiments had indicated produces an unusual sensory irritation of the throat. In experiment 1 subjects swallowed aqueous solutions of ibuprofen prepared with different buffering agents and gave ratings of irritation and taste in the mouth and throat. The results showed that ibuprofen irritates the throat much more than the mouth, and that its quality in the throat is characterized primarily as sting/prick, itch and tickle (often leading to cough). Based upon the results obtained with the different buffering agents, we hypothesized that the sting/prick/itch qualities of throat irritation were pH-dependent. Parametric manipulation of solution pH in experiment 2 confirmed this hypothesis. The same experiment revealed that, in contrast to other oral irritants (e.g. capsaicin and menthol), repeated stimulation caused neither sensitization nor desensitization of throat irritation. In the final experiment we found that ibuprofen's throat irritation could not be modulated by temperature, as it should be if stimulation occurred via capsaicin-sensitive receptors. We therefore conclude that ibuprofen has novel chemesthetic properties, which are not mediated by capsaicin-sensitive (vanilloid) receptors, and that a major component of the throat irritation it produces occurs via a pH-dependent receptor mechanism.

Introduction

Most of what we know about oral sensitivity to chemical irritants is based on studies with capsaicin and a small number of other well-known agents (e.g. menthol, CO₂), all of which have strong sensory effects in the mouth. Although it has been assumed that sensory irritants stimulate the pharynx and throat as well as the mouth, the relative inaccessibility of these caudal oral areas has discouraged studies of their sensitivity. In the only prior psychophysical investigation of chemesthesia in the throat, Rentmeister-Bryant and Green (Rentmeister-Bryant and Green, 1997) had subjects swallow solutions of capsaicin and report on the intensity of sensory irritation in both the mouth and the throat. Capsaicin yielded higher irritation ratings in the throat than in the mouth, and piperine produced similar ratings in the throat and mouth. These findings indicate that the throat might be at least as sensitive to chemical irritants as the mouth, and that for some irritants the throat may be the dominant site of stimulation.

It was in the context of Rentmeister-Bryant and Green's results that we decided to study ibuprofen [2-(4-isobutylphenyl)propanoic acid] as a chemesthetic stimulus. It had been brought to our attention that when swallowed in liquid analgesic formulations, this common non-steroidal

anti-inflammatory drug (NSAID) often triggered unpleasant tastes (usually described as bitterness) and irritation toward the back of the mouth and throat. Moreover, unlike capsaicin and other irritants, ibuprofen seemed to cause no significant irritation in the front of the mouth, and in the throat, sensations of burning and stinging were accompanied by sensations of 'itch' or 'tickle' that sometimes triggered coughing. These unusual characteristics suggested that ibuprofen's sensory irritancy might occur via different sensory mechanisms than the other irritants. Thus, ibuprofen offered a unique opportunity both to study chemesthesia in the throat and to determine if its irritancy might be a novel form that did not involve capsaicin-sensitive (i.e. vanilloid) receptors.

Pilot experiments with aqueous suspensions of ibuprofen quickly confirmed that ibuprofen triggered pronounced throat irritation with only minor mouth irritation, and that the throat irritation was qualitatively different than that produced by irritants such as peppers, mustards or menthol. We therefore undertook a series of psychophysical experiments (i) to specify the location and qualitative profile of ibuprofen's oral and pharyngeal chemesthetic effects, and

(ii) to test hypotheses about the biophysical factors that might underlie these effects.

Experiment 1

Evaluative study and effects of bicarbonate salts

We began by evaluating three dimensions of ibuprofen’s sensory properties: its sensory quality, the sites in the oral–haryngeal region at which it was perceived, and its time-course. Subjects were asked to rate perceived irritation in the mouth and throat and perceived taste intensity using a time–intensity procedure. In separate sessions, subjects were also instructed to indicate, over time, the presence or absence in the mouth and throat of several qualities of irritation (see Table 1) and taste.

In addition, because ibuprofen was thought to produce bitterness along with irritation, and did so in some pilot subjects, and because salts tend to suppress bitterness (Bartoshuk, 1977; Breslin and Beauchamp, 1995, 1997), we initially presented ibuprofen as either a neutralized salt of the acid or mixed with bicarbonate salts. We chose to use large anion salts, like bicarbonate (HCO_3^-), because they have the added benefit of tasting less salty than small ion salts while maintaining bitterness blocking efficacy (DeSimone and Price, 1976; Weiffenbach and Ryba, 1993; Breslin and Beauchamp, 1995). This occurs because the bitterness blocking efficacy of oral sodium salts is a peripheral physiological effect rather than a central cognitive one; and therefore it is independent of the intensity of perceived saltiness (Breslin and Beauchamp, 1995). Finally, to determine if potential interactions were cation-specific, we mixed sodium ibuprofen with sodium bicarbonate, and potassium ibuprofen with potassium bicarbonate.

Materials and methods

Subjects

The eighteen subjects (11 women and seven men) ranged in age from 20 to 40 years and were paid to participate in this study. Subjects were instructed not to eat anything for 2 h prior to testing and were asked about their most recent use of oral irritants (e.g. toothpaste, mouthwash, black pepper, chili pepper, onions and garlic, mustard, ginger, etc.) and analgesics. Subjects having consumed irritants or analgesics within the last 4 h were excused for that day and rescheduled. This screening procedure was followed throughout the study.

Materials/stimuli

The following three ibuprofen solutions were prepared in deionized water: 1% (w/v) (43.8 mM) ibuprofic acid sodium salt (Sigma), pH 7.1; 1% ibuprofic acid sodium salt with 240 mM sodium bicarbonate added, pH 8.15; 43.8 mM ibuprofic acid potassium salt with 240 mM potassium bicarbonate added, pH 8.3. A fourth stimulus containing 1% (w/v) (43.8 mM) ibuprofic acid sodium salt and 240 mM

Table 1 Definitions of sensory irritation quality descriptors employed in experiment

Irritation quality descriptors	
Burning	the sensation that commonly results from exposure to very high temperatures (i.e. thermal burns), skin abrasions (e.g. rug or floor burns) or chemical irritants (e.g. alcohol); may or may not be accompanied by a thermal sensation
Stinging/pricking	sharp sensations similar to those produced by an insect bite— <i>other than itching</i> —or a pinprick; may be constant (stinging) or brief (pricking)
Itching	the sensation that provokes the desire to scratch
Tingling	a lively pins-and-needles sensation
Warm/hot	sensations of mild (warm) or extreme (hot) heating
Numbness	the diffuse (e.g. ‘fuzzy’) sensation produced during the onset of an anesthetic (e.g. novocaine); it is NOT the complete absence of sensation
Tickle	the sensation in the back of the mouth or throat that when weak causes the urge to clear the throat and when strong causes coughing

sodium gluconate was prepared for the training session. The pH was determined using a pH meter (Jenco Electronics, micro-computer pH meter) with a Whatman double junction electrode.

Practice session

Subjects were first given an abbreviated practice session in which they were instructed in the use of a computerized version of the Labeled Magnitude Scale—LMS (Green *et al.*, 1993, 1996). They were also familiarized with the qualitative terms and their definitions (Table 1). To give subjects experience with the rinse and swallow procedure and to familiarize them with the ‘background’ sensations produced by swallowing in the absence of ibuprofen, subjects began by sipping, gently swishing for 10 s and then swallowing 10 ml of deionized water. They then sipped, gently swished for 10 s and swallowed a 10 ml sample of 1% ibuprofic acid sodium salt with 240 mM sodium gluconate added. (Gluconate was used so that subjects would not receive practice with the same salt to be tested experimentally.) Subjects were asked every 30 s for 3 min to rate the intensity of (i) irritation in the mouth and throat and (ii) taste. Subsequently they sipped another stimulus and were asked (over another 3 min interval) to indicate the presence of irritation or taste qualities by checking the appropriate categories on a form sheet.

Experimental procedure

There were 14 experimental sessions. In half of the sessions subjects indicated the presence of taste and irritation qualities in the mouth and throat, and in the other half they rated taste and irritation intensities in the mouth and throat. Ratings were made every 30 s for 3 min, and every 60 s thereafter up to 20 min. Only one 10 ml stimulus, preceded by a single oral rinse with deionized water, was presented per session. Each stimulus was presented twice in each rating condition (i.e. for intensity and quality). The orders of both stimulus presentation and the type of rating were counterbalanced across subjects. The last two sessions were control sessions that consisted of intensity and quality ratings of deionized water. One subject who rated sensation from deionized water above 'weak' on the LMS was dropped from the study and replaced. Two subjects were also removed from the study for highly irregular ratings across replications based upon a rejection criterion of >300% variance between the first time-0 rating and its replication in another test session.

Analysis

Log-means of taste and irritation intensities were calculated because the data were log-normal when plotted as a residuals histogram. An analysis of variance (ANOVA) was run on both the intensity and the quality data. Mouth and throat irritation data were analysed together and separate analyses were carried out for the taste quality data. All post-hoc pairwise comparisons were conducted with Tukey's Honestly Significant Difference test (HSD). The details of the analysis were as follows.

Intensity. A four-factor ANOVA [stimulus (3 levels) \times type of rating (3 levels) \times time (23 levels) \times replication (2 levels)] was conducted on the logged intensity data.

Quality. Because the ratings were scored as zeros and ones to indicate the absence or presence of a particular quality, we could not conduct an ANOVA on the data in their original form. To create a range of response values the scores were pooled over three time-periods: 1–3 min; 9–11 min; and 18–20 min. Two ANOVAs were conducted, one for the irritation quality frequencies and one for the taste quality frequencies. Repetition was removed as a factor and the sum of the two trials was calculated and then summed across the appropriate time period. Irritation qualities were analysed with a 3-way ANOVA [stimulus (3 levels) \times quality (8 levels) \times time (3 levels)], as were taste qualities [stimulus (3 levels) \times quality (5 levels) \times time (3-levels)].

Results and discussion

Intensity of irritation and taste

Ibuprofen alone. Geometric means of the intensity ratings for irritation and taste are plotted in Figure 1. The data have been plotted to show their relationship to scale values on

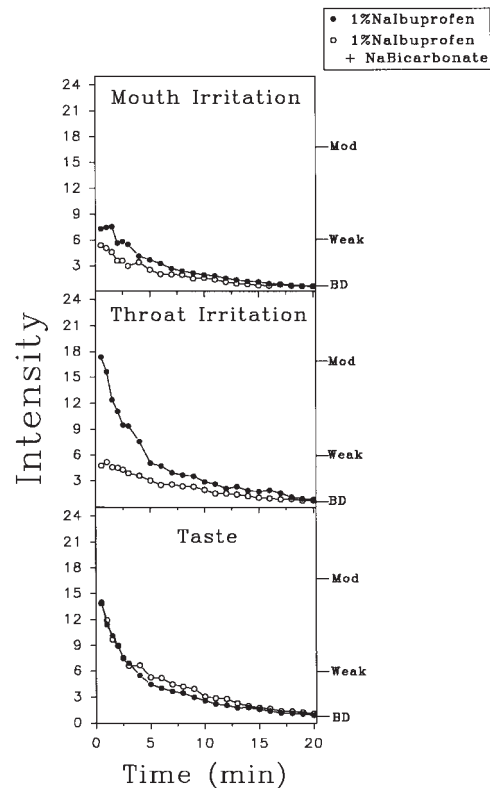


Figure 1 The geometric mean intensity of irritation elicited by 1% sodium ibuprofen solutions (filled circles) in the mouth (top panel) and throat (middle panel) is shown over the 20 min rating period. The bottom panel depicts the intensity of taste sensations. The open circles show the effect of mixing sodium bicarbonate in with 1% sodium ibuprofen. Here and elsewhere, BD = barely detectable, Mod = moderate on the LMS.

the LMS. Ibuprofenic acid sodium salt elicited irritation in the mouth and throat, as well as taste sensations. All three ratings reached a peak within the first minute and declined over time in a roughly exponential manner, becoming 'barely detectable' by the end of the 20 min rating period [$F(22,374) = 17.19$, $P < 0.01$]. Throat irritation reached a 'moderate' level and was more than twice as great as oral irritation [$F(2,34) = 4.94$, $P < 0.05$]. Taste intensity was similar in intensity to throat irritation.

Ibuprofen with bicarbonate. Because the results of mixing sodium bicarbonate and potassium bicarbonate with sodium ibuprofen and potassium ibuprofen (respectively) were similar, only the sodium bicarbonate mixture solutions are depicted in Figures 1–3. Both of the salt-ibuprofen mixture solutions were rated as less irritating in the mouth and the throat than was ibuprofen alone [$F(4,68) = 3.18$, $P < 0.05$] (Figure 1). However, adding the bicarbonates suppressed initial throat irritation by ~76%, whereas initial mouth irritation was reduced only 25%. Post-hoc analyses (Tukey HSD tests) of the three-way interaction among time, location and solution revealed significant differences between 1% sodium ibuprofen and the bicarbonate solutions in the throat through the first 6 min ($P < 0.05$).

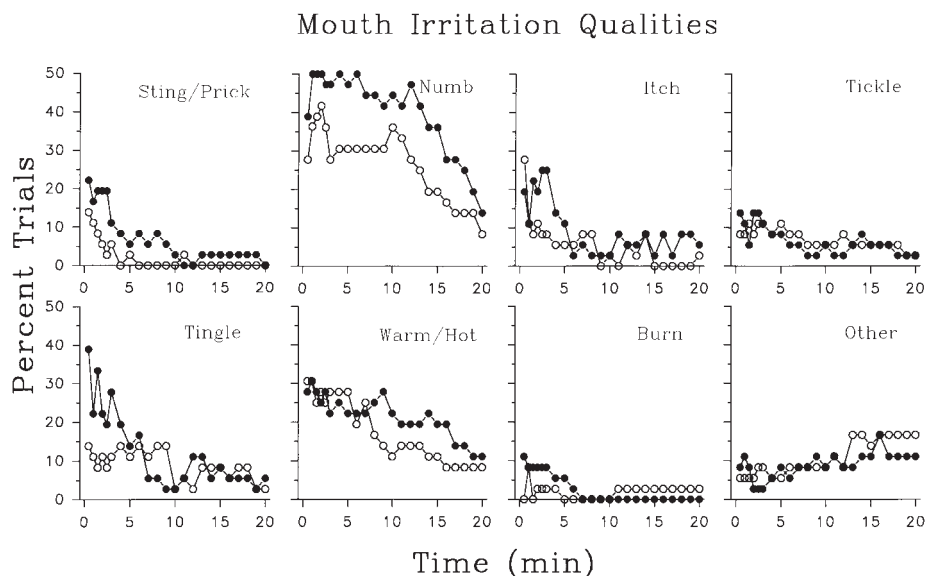


Figure 2 Shown are the frequency of reports of eight qualities of irritation in the mouth. The top row are the mouth irritation qualities: sting/prick, numb, itch and tickle; the bottom row are the qualities: tingle, warm/hot, burn and other (see Table 1 for definitions). The responses were measured over the 20 min rating period. The filled circles depict frequency of quality reports for 1% sodium ibuprofen and the open circles depict 1% sodium ibuprofen mixed with sodium bicarbonate. Note that these measures do not directly reflect the intensity of the sensations.

Surprisingly, the salts had only a weak effect on the taste of ibuprofen. Sodium bicarbonate did not alter overall taste intensity and the potassium bicarbonate enhanced taste intensity by ~14%. The taste of the solutions did not differ significantly and all ratings had declined significantly by the second minute ($P < 0.05$).

The subtle changes in the taste of ibuprofen caused by adding salts were most likely due both to the taste of the added salts themselves as well as cation-specific enhancements or suppressions of the taste of ibuprofen (see taste qualities below). Because it was not obvious why the addition of salts should suppress the irritation of ibuprofen, possible explanations were tested in experiment 2 (below).

Qualities of irritation and taste

Ibuprofen alone. During the first few minutes the most frequently reported qualities in the mouth were numbness and tingle, which soon after exposure were reported in almost 50% and 40% of the trials, respectively (Figure 2). However, reports of numbness did not begin to abate for 10 min or longer, whereas reports of tingle dropped to ~20% after the first minute. Warm/hot, the next most common sensation, fell between numbness and tingle in both frequency and time course, and all other qualities were reported less often. Note that no quality was reported in more than half of the trials, which means that oral irritation from ibuprofen is complex.

Figure 3 shows that the throat yielded a different quality profile than the mouth [$F(7,119) = 35.01$, $P < 0.01$]. In the first few minutes, tickle was reported in the throat on 35–50% of trials, with sting/prick occurring nearly as often

but declining more quickly. Itch was almost as common as sting/prick, reaching 40% by 1.5 min, and tended to last longer. Numbness, tingle, warm/hot and burn all initially were reported in >30% of the trials. Reports of other qualities occurred <5% of the time.

Post-hoc analyses (Tukey HSD tests, $P < 0.05$) showed that tickle was the only quality for which the overall frequency of reports differed between the mouth and throat, but there appeared to be a tendency for sting/prick to be reported more often early on in the throat compared to the mouth. In the mouth, numbness was reported significantly more often than all qualities except warm/hot. These data confirm reports that sensitivity to chemical irritation is both compound and location-specific in the mouth and pharynx (Lawless and Stevens, 1988; Rentmeister-Bryant and Green, 1997).

Figure 4 shows that the taste of sodium ibuprofen was dominated by bitterness and saltiness early, with sweetness tending to develop after several minutes. All four taste qualities decayed in frequency after the first 5–10 min and there was a significant interaction between quality and time [$F(8,136) = 24.34$, $P < 0.01$]. Reports of both bitter and salty decreased over time, whereas reports of sweet first increased then decreased. The induction of sweetness was unaffected by the addition of salts.

Ibuprofen with bicarbonate. In the mouth, the most noticeable effects of adding sodium bicarbonate were to suppress tingle and numbness, but all qualities except 'other' were also reduced to some degree. In the throat, addition of bicarbonate salts virtually eliminated some qualities, such

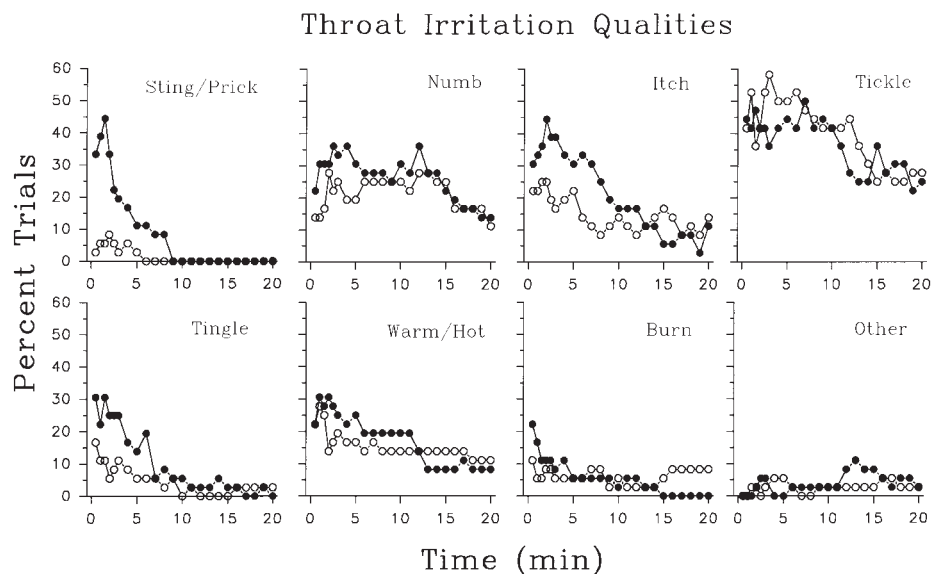


Figure 3 Shown are the frequency reports of the eight qualities of irritation in the throat. The top row are the throat irritation qualities: sting/prick, numb, itch and tickle, and the bottom row are the qualities: tingle, warm/hot, burn and other (see Table 1 for definitions). The filled circles depict frequency of quality reports for 1% sodium ibuprofen and the open circles depict 1% sodium ibuprofen mixed with sodium bicarbonate. Conditions under which these data were collected were the same as for Figure 2.

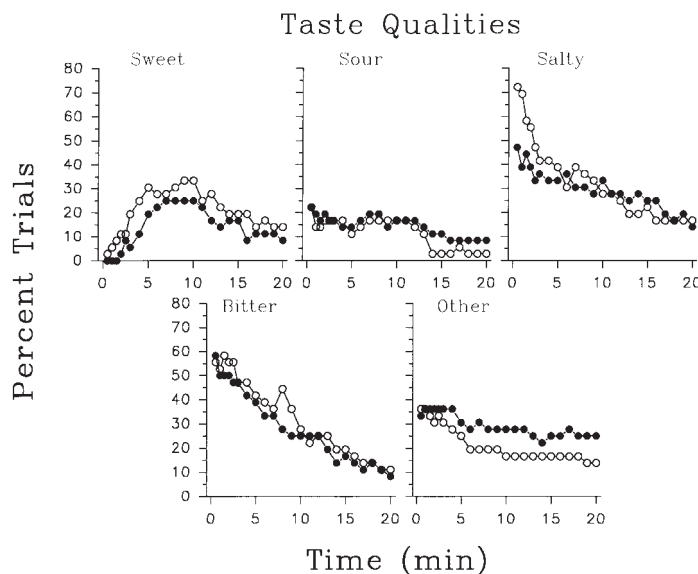


Figure 4 The frequency reports of the five taste qualities are shown over the 20 min rating period. The filled circles depict frequency of quality reports for 1% sodium ibuprofen and the open circles depict 1% sodium ibuprofen mixed with sodium bicarbonate. These measures do not directly reflect the intensity of the qualitative sensations. Note that sweet responses were initially zero and increased in frequency during the middle of the rating period.

as sting/prick and burn, while leaving other qualities unaffected (Figure 3).

Taste qualities were affected only a little by bicarbonate salts (Figure 4), with slight tendencies to increase reports of sweet and salty, and to decrease reports of 'other' [$F(8,136) = 4.48$, $P < 0.05$]. The increase was greatest for saltiness, consistent with the addition of sodium. It should be noted that the failure to reduce the frequency of reports of bitter-

ness does not necessarily mean that the intensity of bitterness was not reduced.

Because the intensity of taste was surprisingly high compared to irritation, we speculated that subjects might have attributed some of the salient odor of ibuprofen to taste. An informal study conducted with the nares pinched closed to block the odor of ibuprofen indicated that taste was perceived less often and less intensively under those

conditions. It is therefore likely that the odor of ibuprofen in solution led to an overestimate of the taste of the solutions. In subsequent experiments we avoided this complication and focused our attention on the unusual throat irritation from ibuprofen.

Experiment 2: effects of cation, pH and repetition

In experiment 1 the addition of bicarbonate salts significantly reduced the overall intensity of throat irritation, and appeared to have their greatest effect on the qualities of sting/prick and itch. This effect was not related to the specific cation that was used, since sodium and potassium had equally suppressive effects overall. It was also unlikely to have been due to the taste of salts interacting with perceived irritation, since neither of the bicarbonate salts has a strong taste. The suppression was more likely caused by a chemical property of the anion. The two properties that were the best candidates were (i) the buffering capacity and pH-raising effects of bicarbonate ions and (ii) the ability of bicarbonate ions (i.e. CO_2) to diffuse passively or be transported across membranes into cells (Laiken and Fanestil, 1985). This latter process could have a significant impact on intracellular pH due to the CO_2 carbonic acid bicarbonate equilibrium. To determine which possibility could better account for the decrease in irritation, we used glycineamide—which is less likely to diffuse as freely into cells (Sussan *et al.*, 1999) as bicarbonate—to buffer ibuprofen solutions to the same pH as we had with bicarbonate. If glycineamide failed to reduce irritation, we could conclude that the effect of bicarbonate was due in part to diffusion of ions through cell membranes. If glycineamide did decrease irritation, then bicarbonate's effects would be more likely due directly to a change in pH.

The experiment was also designed to evaluate the effect of repeated exposures to ibuprofen. This allowed us to assess the within-subject reliability of irritation ratings as well as the potential for ibuprofen irritation to either sensitize or desensitize over time in the manner of capsaicin and other irritants (Carpenter and Lynn, 1981; Green, 1989, 1991; Cliff and Green, 1994; Prescott and Stevenson, 1996a,b; Prescott, 1999).

Materials and methods

Subjects

Sixteen subjects (nine women and seven men, aged between 20 and 40 years) participated in this study, 12 of whom had participated in experiment 1.

Stimuli

There were five stimuli. All contained 1% ibuprofenic acid (w/v; 48.5 mM) and 1% glycineamide HCl (90.5 mM), which also acted as a buffer (the pK_a of glycineamide is 8.1). Solutions were buffered to a pH of 7.2, 8.2 and 9.2 using NaOH, and to a pH of 8.2 and 9.2 using KOH. The KOH

solution buffered to pH of 8.2 was used during training only. These pH values were selected because the average pH of the bicarbonate solutions in experiments 1 and 2 was 8.2 (the pK_a of sodium bicarbonate is 6.28). Therefore, the glycineamide HCl solutions were centered on a pH of 8.2 and moved up and down one log step to test for the effects of pH on irritation. The pH 9.2 KOH solution was used as a control for the cationic effects of sodium in the pH 9.2 NaOH solution, when sodium concentration would be the highest and most likely to have an effect.

Practice session

During training subjects were first asked to swallow two mouthfuls of deionized water and then to swallow a 10 ml sample of the ibuprofen/glycineamide solution buffered to pH 8.2 with KOH. They were not to swish it, but to swallow it directly and to rate only the intensity of irritation in the throat (using the LMS) at times 0, 15 s, 30 s, 1 min, 2 min and 3 min. Only throat irritation was rated because experiment 1 had indicated that the throat was the primary site of irritation. Similarly, ratings were recorded only during the first 3 min because this time period had the highest irritation ratings (Figure 1). Subjects were instructed to swallow just prior to each rating because the mechanical stimulation of swallowing appeared from pilot work to enhance irritation. There was then a 2 min time-out before the process was repeated twice, each time with a new 10 ml sample. The total rating time per practice session was 13 min (2 min pause between each of the 3 min tests).

Procedure

Subjects were divided into two groups that received either the NaOH stimuli with a U-shaped pattern of descending then ascending pH (9.2, 8.2, 7.2, 8.2, 9.2), or the opposite pattern of ascending then descending pH. The same solution was administered three times on a given day and all subjects were tested with each of the four solutions twice (requiring eight sessions). For both groups the stimulus delivered on the fourth and eighth sessions was the solution buffered to a pH of 9.2 with KOH (e.g. day 1, 9.2 Na; day 2, 8.2 Na; day 3, 7.2 Na; day 4, 9.2 K; day 5, 7.2 Na; day 6, 8.2 Na; day 7, 9.2 Na; day 8, 9.2 K). On any given day the subjects proceeded as they had during practice: they first swallowed two mouthfuls of deionized water, then a 10 ml sample of the solution, and rated throat irritation for 3 min. After a 2 min pause they repeated the sequence (without water rinse) twice more.

Analysis

Log means were calculated for statistical purposes and geometric means were plotted. Data were analysed with a four-way repeated measures ANOVA on the logged values [solution (4 levels) \times time (6 levels) \times repeated trials (3 levels) \times replication (2 levels)].

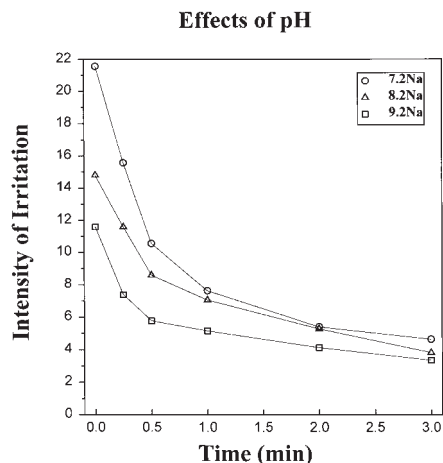


Figure 5 Depicted here is the impact of solution pH on the geometric mean perceived throat irritation from ibuprofen. Three solutions were 1% sodium ibuprofen with glycinate added and adjusted to pH 9.2 (squares), pH 8.2 (triangles), and pH 7.2 (circles) by the addition of NaOH.

Results and discussion

Effects of pH on irritation

The effects of pH on throat irritation can be seen in Figure 5. Basically, the lower the pH (the more neutral solution) the more irritating ibuprofen was perceived to be [$F(1,15) = 4.93$, $P < 0.05$], with initial irritation almost twice as high at the lowest pH as at the highest pH. Because all three solutions had the same ibuprofen concentration and same buffer concentration, pH was the only factor that could have affected the level of irritation.

Irritation decayed significantly [main effect, $F(5,75) = 26.73$, $P < 0.01$] and the rate of decay was greatest at the lowest pH [time \times stimulus interaction, $F(15,225) = 1.97$, $P < 0.05$]. The sodium-buffered solutions revealed significant differences between pH 7.2 and pH 8.2 in the first minute; pH 7.2 and pH 9.2 at all time points were significantly different; and pH 8.2 and pH 9.2 revealed significant differences at all time points with the exception of the first and last time points (Tukey HSD, $P < 0.05$).

Effects of cation

Figure 6 shows the irritation ratings for the two pH 9.2 solutions, one with NaOH and the other with KOH. The results confirm the finding of experiment 1 that the cation associated with the base was not a significant factor in determining irritation. The irritancy of the two cations was virtually indistinguishable. Once again, pH appeared to be the key factor affecting perceived irritation.

Individual differences

We had noticed in the course of pilot testing and in experiment 1 that the variability among subjects in their sensitivity to ibuprofen was large. While some reported strong irritation, others reported none. To test whether these differences were reliable we selected three subjects who were 'high

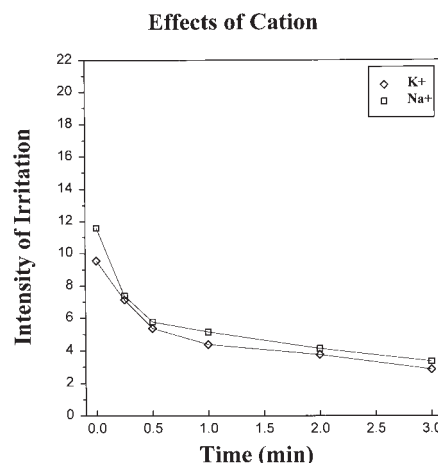


Figure 6 The impact of cations on geometric mean perceived irritation from ibuprofen localized in the throat is shown for two solutions: 1% sodium ibuprofen with glycinate added and adjusted to pH 9.2 (square) by the addition of NaOH; 1% potassium ibuprofen with glycinate added and adjusted to pH 9.2 by the addition of KOH (diamond).

responders' and three who were 'low responders' based on their response to the first exposure to ibuprofen at pH 7.2, then plotted their responses during the first trial for all four stimuli. Figures 7 and 8 show that although 'high responders' reported much more irritation for all four stimuli than did 'low responders', both groups showed the same relative effects of pH (the y-axis is expanded in Figure 8 to amplify the 'low responders' curves). Low responders differed in sensitivity and not merely in scale usage. For example, we were unable to elicit even moderate irritation from low responders with an ibuprofen concentration as high as 10% (w/v).

Effects of repeated stimulation

There was no compelling evidence of sensitization or desensitization of perceived irritation from repeated stimulation. Only the pH 7.2 solution showed a slight (non-significant) trend toward sensitization over successive trials. The failure to observe effects of repeated stimulation under these conditions does not rule out the possibility that they occur at higher concentrations, or when no additional buffer is used.

Experiment 3

Effect of temperature on pharyngeal irritation from ingested ibuprofen

Because several well-studied irritants interact strongly with temperature [e.g. capsaicin (hot) and menthol (cool)], and because vanilloid receptors sensitive to capsaicin (VR1s) are temperature-sensitive (Caterina *et al.*, 1997), we wished to determine whether ibuprofen's irritancy could also be modulated by temperature. In other words, does ibuprofen produce sensory irritation by stimulating temperature-sensitive nociceptors in the manner of either capsaicin or menthol (Green, 1986; Tominaga *et al.*, 1998)?

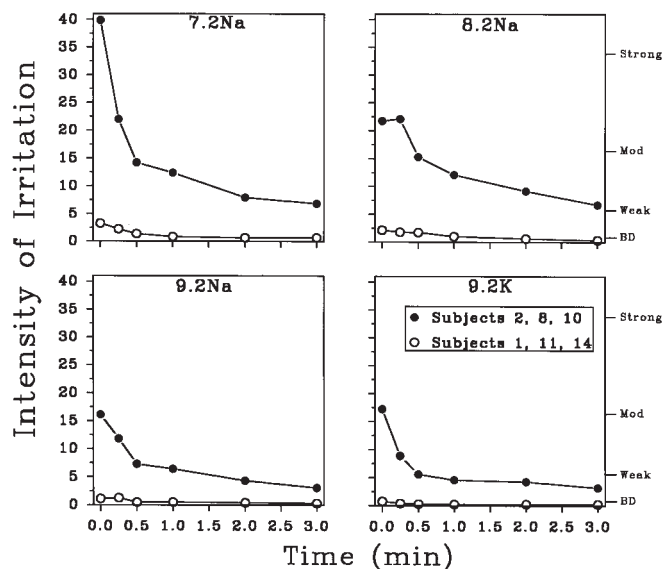


Figure 7 Replotted here are a portion of the data from Figures 5 and 6 comparing the three most sensitive subjects (filled circles) and the three least sensitive subjects (open circles). The top left panel shows pH 7.2 data, the top right shows pH 8.2 and the bottom left shows pH 9.2 (all three were NaOH neutralized) and the bottom right shows pH 9.2, KOH neutralized.

Materials and methods

Subjects

Seventeen people (ten women and seven men, aged between 20 and 40 years) who had participated in at least one of the previous ibuprofen experiments, participated in the present one.

Stimuli

The stimuli were 10 ml volumes of 1% sodium ibuprofen (w/v; 43.8 mM) at a pH 7.1 presented at four different temperatures: 6, 25, 42 and 60°C. Deionized water alone was presented at the same temperatures to control for the perception of temperature *per se*. The order of testing was counterbalanced for stimulus and temperature.

Procedure

The four ibuprofen and four water stimuli constituted eight conditions. Subjects swallowed a solution and rated the intensity of the sensation of irritation in the throat (using the LMS) during and at times after the swallow: 15s, 30 s, 45 s, 1 min, 2 min and 3 min. For every rating, subjects were instructed to rate throat irritation perceived during swallowing. One subject was dropped from the experiment because he rated irritation from deionized water at 25°C to be greater than 'weak'.

Analysis

As before, the data were normalized by computing logarithms, and geometric means were plotted. A four-way ANOVA was conducted on the log values [solution (2 levels)

Subject #'s 1, 11, 14

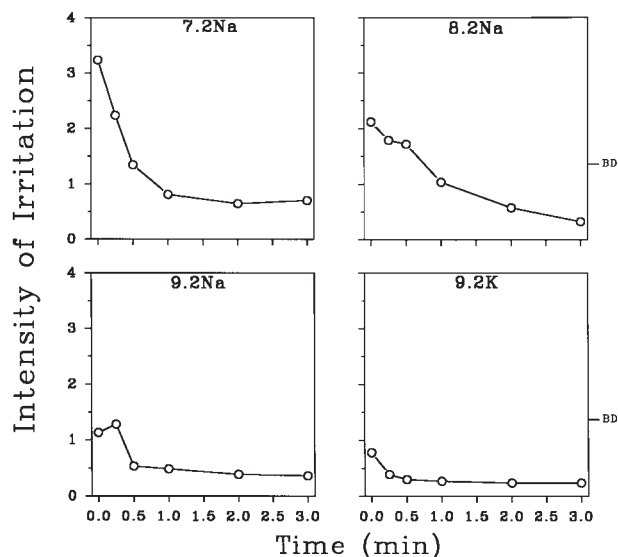


Figure 8 The data of Figure 7 are displayed with the y-axis expanded 10-fold to show the pH and cation trends in the data for the three least sensitive subjects.

× temperature (4 levels) × time (7 levels) × replication (2 levels)] on the log values.

Results and discussion

Figure 9 shows that sensory irritation from ibuprofen was unaffected by temperature. There was a slight tendency for ibuprofen to be less irritating at 60°C than at the three cooler temperatures, but this trend was not significant. We can infer from this result that ibuprofen does not produce its sensory irritation via temperature-sensitive transduction pathways. This appears to rule out an involvement of VR1s as mediators of any of ibuprofen's chemesthetic effects, including sting/prick, itch and tickle.

General discussion

The sensory characteristics of ibuprofen appear unlike those of any other sensory irritant so far studied (see Figure 10 for ibuprofen's structure). The most notable of these characteristics is that its irritancy is much more pronounced in the throat, where its quality is a complex combination of stinging, pricking, itching and tickling. In stark contrast to the pungency of capsaicin and other irritants, ibuprofen's lesser effects in the mouth are primarily tingling and numbness. Indeed, numbness and tingling are typically associated with a loss of sensitivity (i.e. anesthesia) rather than with stimulation.

Ibuprofen also elicited taste sensations that were most often described as bitter or salty. We subsequently found that pinching the nares reduced the perceived intensity of ibuprofen's taste, which suggests that ibuprofen has an odor that is referred to the oral cavity as taste. However, referred

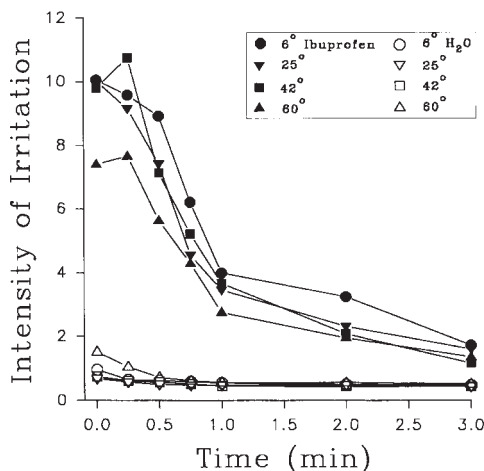


Figure 9 The effects of solution temperature on the geometric mean ratings of sensory irritation in the throat are shown for 1% sodium ibuprofen (filled symbols) and deionized water (open symbols) at four temperatures. Ratings were made over a period of 3 min.

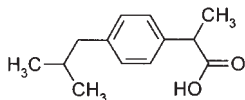


Figure 10 The structure of ibuprofen [2-(4-isobutylphenyl)propanoic acid] is depicted.

olfactory sensations cannot explain the reports of sweetness that occurred several minutes after ibuprofen had been swallowed. This long-latency sweetness deserves further study, as it implies that (in some individuals) ibuprofen may have a delayed excitatory effect on sweet-sensitive taste receptors.

Individual differences

Individuals differed greatly in responsiveness to ibuprofen. While some subjects reported its throat irritancy to be 'barely detectable', others reported it to be 'strong' or 'very strong'. In addition, not all subjects reported the same qualitative profile of sensations. For some, ibuprofen was difficult to swallow without coughing, while for others stinging and pricking were more noticeable than itching and tickle. The source (or sources) of these individual differences is not clear, although there are at least four possibilities: (i) differences in the depth of the nociceptors that are sensitive to ibuprofen; (ii) differences in the total number of nociceptors sensitive to ibuprofen; (iii) differences in the structure/sensitivity of the nociceptors (polymorphisms); and (iv) differences in epithelial/salivary composition that affect the availability of ibuprofen to receptors. In view of the pH-sensitivity of ibuprofen irritation, we are currently investigating the last possibility to see if variations in salivary buffering capacity may contribute to individual differences in sensitivity.

Temperature independence

The temperature independence of ibuprofen's irritancy contrasts with the extent to which temperature modulates sensory irritation from other well-known irritants, such as capsaicin (Szolcsanyi, 1977; Carpenter and Lynn, 1981; Green, 1986) and methyl salicylate (Green and Flammer, 1989), and suggests that ibuprofen does not stimulate capsaicin-sensitive (or other thermally labile) nociceptors, such as VR1s (Caterina *et al.*, 1997). This inference finds additional support in the low frequency of reports of burning (as opposed to stinging) from ibuprofen. Heat-sensitive C-fibers are believed to mediate burning heat pain (Torebjork, 1974; Torebjork *et al.*, 1984) and are the likely candidates to mediate burning from irritants as well. Ibuprofen therefore appears unusual in that it may stimulate nociceptors that respond to chemicals, and perhaps to mechanical stimulation, but not to temperature.

Temporal factors

Ibuprofen also differs from capsaicin and other irritants in that it shows little effect of repeated stimulation, i.e. neither sensitization nor desensitization (Green, 1989, 1991). The lack of cumulative effects of stimulation also suggests that ibuprofen does not build up significantly in the mucosa during repeated stimulation, which would be expected to produce growing irritancy across trials. The possibility cannot be ruled out, however, that temporal effects would emerge if stimulation were delivered at either shorter inter-stimulus intervals or over longer periods of time.

Possible mechanisms of action

Ibuprofen's ability to cause sensory irritation does not seem related to its analgesic properties, which are thought to be indirect and associated with its anti-inflammatory activity. Specifically, ibuprofen has been shown to inhibit cyclooxygenase (i.e. prostaglandin endoperoxide synthase), which in turn prevents synthesis of prostaglandins (Insel, 1996). Because prostaglandin E₂ has been implicated as a sensitizing agent for some nociceptors (Pitchford and Levine, 1991; Davis *et al.*, 1993; Lopshire and Nicol, 1997), most notably VR1s in the presence of protons (Vyklíckí *et al.*, 1998), blocking its synthesis should make it more difficult to stimulate these nociceptors rather than easier. It is therefore paradoxical that ibuprofen appears to have an excitatory effect on at least some mucosal nociceptors (presumably non-VR1s) when it reaches them more or less directly through the mucosal epithelium.

The potent effect of pH on ibuprofen's irritancy suggests that it stimulates a subset of pH-sensitive pain fibers (Rang *et al.*, 1991; Steen *et al.*, 1992; Bryant and Moore, 1995). It is possible that ibuprofen interacts with a vagal pH-sensitive nociceptor other than VR1 (Chen *et al.*, 1997; Seno *et al.*, 1998). Exactly what type of nociceptors these are and how they are stimulated remains to be learned. The evidence

that buffers and lower pHs increase ibuprofen irritation, however, only indicates that some of ibuprofen's effects are pH-dependent, not necessarily that protons themselves are critical to the transduction process. At lower pH (towards neutrality) ibuprofen is more protonated, but it is also more lipid-soluble. Increased lipophilicity would facilitate mucosal absorption of ibuprofen and, hence, improve its access to nociceptive fibers and also to intracellular processes, regardless of the mechanism of nociceptor activation. The present data cannot be used to rule out possible effects of stimulus lipophilicity and/or proton delivery. To do this may require *in vitro* experimental evidence that ibuprofen was impacting nociceptor activity independently of its pH.

A third potential mechanism that can be ruled out is that ibuprofen's excitatory effects depend upon delivering protons to an epithelial-surface receptor. Ibuprofen is not perceived to be sour (nor is any acid at pHs > 7) and common organic food acids that do deliver protons to the epithelial surface (e.g. citric, tartaric and malic acids) do not elicit ibuprofen's signature throat irritation at any pH, even though these acids could carry the same number of protons as ibuprofen (Petrucci, 1982). In addition, the more lipophilic food acids such as benzoic acid and its derivatives also evoke different qualities of irritation than ibuprofen (Peleg and Noble, 1995).

Whatever the mechanism is by which ibuprofen has its effects, its ability to produce more prominent and prolonged stinging in the throat than in the mouth, as well as to stimulate itch and tickle in the throat, suggests that the anterior and posterior oropharyngeal regions may possess different arrays of sensory receptors. This possibility is made plausible by the fact that these regions, which serve somewhat different motor and sensory functions, are innervated by different cranial nerves (V, the trigeminal nerve, versus IX and X, the glossopharyngeal and vagus nerves, respectively), and by recent evidence that VR1s are found in the trigeminal nerve but not in the vagus nerve (Caterina *et al.*, 1997). For example, ibuprofen may produce its signature throat irritation by stimulating a class of epithelial pH-sensitive receptors that mediate stinging/pricking sensations, and a subset of mechanically sensitive receptors that play a role in generating tickle and cough.

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