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Real-time Monitoring of Nasal Mucosal pH During Carbon Dioxide Stimulation: Implications for Stimulus Dynamics

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Abstract

Carbon dioxide is a commonly employed irritant test compound in nasal chemesthetic studies because it is essentially free of olfactory stimulus properties. CO_2 is thought to act via hydration to H_2CO_3 and dissociation to H^+ in nasal mucus, with resulting activation of acid sensors. However, transient changes in nasal mucosal pH have not been documented during CO_2 stimulation in humans. We placed a small pH probe on the floor of the right anterior nasal cavity during CO_2 stimulation in eight human subjects with historically high (>30%) and low (≤20%) CO_2 detection thresholds. Three second pulses of CO_2 (15–45% v/v) paired with air in random order (12–15 s inter-stimulus interval; 60 s inter-trial interval) were administered by nasal cannula at 5 l/min. in an ascending series. For each subject, both a CO_2 detection threshold and suprathreshold psychophysical ratings [ψ; labeled magnitude scale] were generated. All subjects showed phasic drops in pH associated with CO_2 stimulation (ΔpH). For all subjects combined, a positive correlation was apparent between applied [CO_2] and both ΔpH and ψ, as well as between ΔpH and ψ themselves ($P < 0.0001$ for each comparison). Subjects with historically low CO_2 thresholds showed steeper dose-response curves for ψ as a function of both applied [CO_2] and ΔpH, but not for ΔpH as a function of applied [CO_2]. For the six of eight subjects with measurable pH changes at threshold, ΔpH was positively related to log [CO_2 threshold] ($P < 0.01$). These data imply that variability in CO_2 detection thresholds and suprathreshold rating may derive from intrinsic differences in neural sensitivity, rather than differences in stimulus activation to hydrogen ion.

Key words: carbon dioxide, nasal mucosal pH, psychophysical testing, sensory irritation

Introduction

Phasic changes in mucosal pH have been presumed to underlie the effectiveness of carbon dioxide (CO_2) as a chemosensory stimulus (irritant) in the upper airway and oral cavity. This presumption is based upon the known chemistry of CO_2's interaction with water, particularly in the presence of the enzyme carbonic anhydrase (Bryant, 2000). Empirical evidence derives from both animal and human experiments in which pharmacological inhibition of carbonic anhydrase results in decreased electrophysiological or psychophysical responses (Dessirier *et al.*, 2000; Alimohammadi and Silver, 2001). In humans, static measurements of nasal mucosal pH have been carried out for the evaluation of various disease states and therapeutic interventions, but have not, to our knowledge, been applied to chemosensory studies (Cavaliere *et al.*, 1988; England *et al.*, 1999; Hehar *et al.*, 1999; Selimoglu *et al.*, 1999; Washington *et al.*, 2000). Here, we determined whether CO_2-induced nasal sensory irritation reflects transient stimulus-related changes in nasal mucosal pH. To address this question, we studied this phenomenon by combining real-time nasal mucosal pH measurements with structured psychophysical testing, including both threshold determination and suprathreshold rating.

Materials and methods

In order to maximize contrasts (i.e. to maximize our statistical power to test hypotheses relating psychophysical to physiological measures), subjects with historically high (>30%) or low (≤20%) CO_2 detection thresholds were studied.

Subject recruitment

Eight non-smoking, non-asthmatic subjects between the ages of 21 and 56 years (including six males and four allergic rhinitics) participated. All were previous participants in chemosensory studies who had demonstrated either consistently high (>30%) or low (≤20%) CO_2 detection thresholds

Table 1 Characteristics of subjects

Historical CO_2 threshold	Gender (M/F)	Age (years, SD)	Allergies (yes/no)	Mean CO_2 threshold 1 (SD)	Mean CO_2 threshold 2 (SD)	Mean CO_2 threshold 3 (SD)
High ($n = 4$)	4/0	47.8 (16.6)	2/2	41.3 (6.6)	38.8 (6.0)	33.8 (3.8)
Low ($n = 4$)	2/2	33.0 (5.2)	2/2	18.1(2.4)	15.6 (1.3)	16.3 (2.5)

on two previous testing sessions at least 2 months apart, utilizing the testing protocol described below (four in each subgroup; Table 1). Subjects' allergy status was confirmed by matching questionnaire responses with results of skin prick testing with a panel of 16 common aeroallergens/mixes; seasonal allergic rhinitic subjects were studied outside of their relevant aeroallergen season. Subjects signed a written informed consent approved by the Committee on Human Research of the University of California, San Francisco prior to testing. Subjects refrained from eating, drinking or exercising for at least 1 h prior to testing, and also refrained from using scented products during the day of testing. Testing took place in a 950 ft^3 climate-controlled chamber maintained at 22 ± 1°C and 40 ± 3% relative humidity.

pH recording

Nasal mucosal pH was measured using a 3 mm diameter flexible glass-tipped probe medically approved for monitoring esophageal pH (Probe M3, Medical Instruments Corporation, Solothurn, Switzerland). After daily calibration (at pH 4.0 and 7.0), the probe was introduced 4 cm along the floor of the right nasal cavity and maintained in position using paper tape applied to the nose and forehead. The probe, in turn, was connected to a direct-reading pH meter with analog output (Model 6171, Jenco Instruments, San Diego, CA). Output from the pH meter was digitized using an analog-to-digital convertor board (DI-195B, DataQ Instruments, Akron, OH) and signal conditioning input module (SCM5B41–01, ±1V, Dataforth Corp., Tucson, AZ) on a Windows-compatible laptop computer (Dell Computers, Roundrock, TX). Data were logged utilizing commercial software (WinDaq, DATAQ Instruments) with a sampling rate of 10 Hz.

CO_2 stimulation

On a single testing date, nasal pH was measured in the right anterior nasal cavity during CO_2 stimulation. The stimulus delivery apparatus and testing protocol have been described in detail previously (Shusterman and Balmes, 1997; Shusterman *et al.*, 2001). Briefly stated, 3 s stimulus pulses, paired with 3 s air pulses in random order (12–15 s interstimulus interval; 60 s inter-trial interval) were administered dichorhinically in a single-blinded fashion by cannula (no. 1606; Salter Labs, Arvin, CA) at 5 l/min, synchronized with inspiration. CO_2 stimuli followed an ascending concentration series (beginning with 0% or 'sham', and progressing to 15, 20, 25, 30, 35, 40 and 45%), with five trials per CO_2 concentration. For each trial, subjects were asked to identify the more irritating of the two pulses, and in addition, to rate the more irritating pulse using a computer-based version of the labeled magnitude scale with the index terms 'none', 'barely detectable', 'weak', 'moderate', 'strong', 'very strong' and 'strongest imaginable' (Green *et al.*, 1996). The CO_2 detection threshold was defined as the lowest concentration at which a subject identified all five CO_2 stimuli as being more irritating than the paired air pulses. Psychophysical ratings of nasal irritation (ψ, 0–100) were also recorded directly to computer file using commercial data acquisition software (LabView, National Instruments, Austin, TX).

Data abstraction and analysis

Delta pH (ΔpH) was defined as any phasic (transient) pH change (exceeding baseline oscillatory fluctuations) registered within 30 s following the onset of a stimulus pulse, and was measured using the 'data calipers' (graphical scaling tool) feature of WinDaq. In addition to noting ΔpH, time latency to maximum pH deviation was recorded, and note was made, when possible, of the mean ΔpH measurable at a particular subjects' threshold CO_2 step. Data were analyzed on a Macintosh G4 computer (Apple Computers, Cupertino, CA) using JMP software (SAS Institute, Cary, NC). Prior to analysis, individual ΔpH and psychophysical data were averaged for the five trials at a given stimulus concentration. Repeated-measures ANOVA was used to evaluate the stability of individual CO_2 detection thresholds over time. CO_2 detection thresholds with and without the pH probe were compared using Student's *t*-test. Linear regression was applied to pooled data (and multivariate regression models examined the role of individual subject) for applied $[CO_2]$ and measured ΔpH, and ψ. Analysis of covariance (ANCOVA) was used to evaluate the effect of historical CO_2 threshold ('high' vs. 'low') on the relationship of $[CO_2]$ to both ΔpH and ψ. Based upon theoretical considerations (Gescheider, 1985), the [natural] log of CO_2 detection thresholds was used to examine the relationship between CO_2 detection threshold and ΔpH at threshold.

The hypotheses to be tested included: (1) pulsed CO_2, but not air, will produce negative phasic changes in nasal pH (ΔpH); (2) the magnitude of ΔpH will be proportional to the CO_2 concentration administered;(3) psychophysical ratings of nasal irritation (ψ) for individual stimuli will be proportional to the magnitude of ΔpH; (4) The relationship of ΔpH to applied $[CO_2]$ will be constant across subjects; (5) The

relationship of ψ to ΔpH will be constant across subjects; (6) ΔpH measured at threshold will be constant across subjects.

Results

Characteristics of participating subjects appear in Table 1. The eight subjects ranged in age from 21 to 56 years (mean 39.4), and included six males and an equal number of allergic rhinitics and non-rhinitics. Mean CO_2 detection thresholds differed between the historically high- and low-CO_2 threshold subgroups in an ANCOVA model including historical testing times 1 and 2 (mean of two determinations each) and at time 3 (single determination along with pH measurement; $P < 0.001$). In terms of stability of measures, there was a downward trend in mean CO_2 detection threshold in the high-threshold group indicative of a possible training effect ($P = 0.17$ in repeated measures subanalysis). However, this effect was overshadowed by measured stability grouping by either historical CO_2 threshold or individual subject ($P < 0.0001$).

Subjects adapted quickly to the presence of the pH probe and tolerated the procedure well. Mean (±SEM) CO_2 detection thresholds with the probe in place did not differ significantly from the immediately previous measurement (25.0 ± 3.8 vs. 27.2 ± 4.6% respectively; $P = 0.72$). With regard to CO_2 detection thresholds, the pH probe was equally unobtrusive in the historically high- and low-threshold subgroups (data not shown). All subjects showed phasic drops in pH associated with CO2—but not air—stimulation (see Figure 1 for representative tracings). Averaged by trial, latencies to peak deviation varied from 2.8 to 12.9 s (mean 7.3; median 6.7), and ΔpH ranged up to 0.26 pH units. Baseline nasal mucosal pH (recorded between stimulus trials) ranged between approximately 7.04 and 7.70 (i.e. physiologically neutral), and did not vary significantly by subjects' CO_2 threshold or rating behavior.

Applying linear regression to data from all subjects combined, a positive relationship was apparent between applied CO_2 concentration and ΔpH ($P < 0.0001$; Figure 2a). Stratified by historical CO_2 sensitivity, the regression lines for the high- and low- threshold groups were parallel (0.0029 pH units/%CO_2) and not significantly displaced from one another ($P = 0.25$; Figure 2b). A multivariate model confirmed that subjects did not differ in their acidification of nasal mucus in response to CO_2 ($P = 0.79$)

For all subjects combined, a positive relationship was apparent between applied CO_2 concentration and ψ ($P < 0.0001$; Figure 3a). Stratifying by historical CO_2 sensitivity, the regression lines for the high- and low-threshold groups had significantly different slopes, with ψ increasing by 0.94 units per %CO_2 in the low-threshold group and 0.48 units per %CO_2 in the high-threshold group ($P < 0.01$; Figure 3b). A multivariate model indicated that individuals differed significantly in their psychophysical response to increasing CO_2 concentrations ($P < 0.0001$).

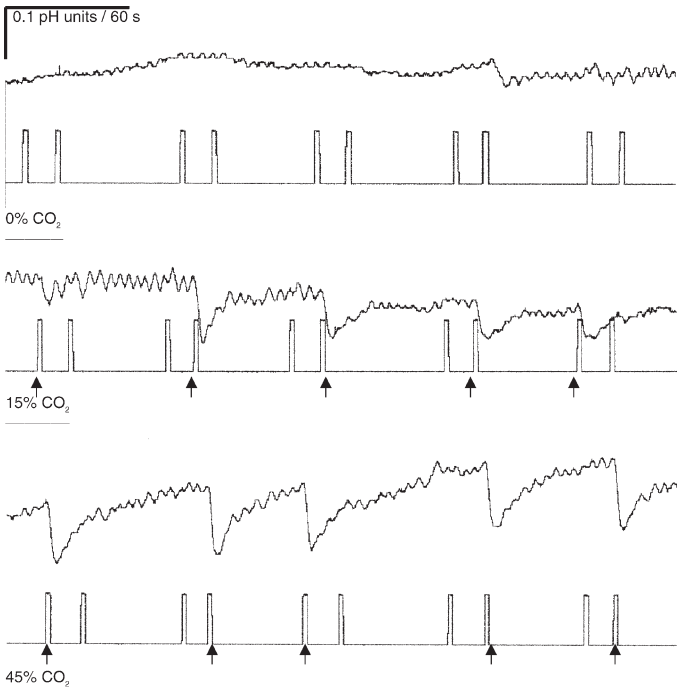

Figure 1 Example of real-time recording of nasal mucosal pH during pulsed stimulation with CO_2 (rectangular waves with arrows) and air (rectangular waves without arrows). Pulses are 5 l/min., 3.0 s duration, synchronized with inspiration, and delivered dichorhinically by nasal cannula. The first series of pulses were all control (0% CO_2) stimuli; vertical arrows in series 2 and 3 indicate CO_2 stimuli. Note baseline shift (as distinguished from phasic change) during control series (probably due to movement artifact). Subject is a 29 year old female.

In addition to their individual relationships to applied CO_2 concentration, ψ and ΔpH, grouped by individual + trial, were themselves significantly correlated ($P < 0.001$; Figure 4a). Stratifying by historical CO_2 sensitivity, the regression lines for the high- and low-threshold groups had significantly different slopes, with ψ increasing by 274 per ΔpH unit in the low-threshold group and 61 per ΔpH unit in the high-threshold group ($P < 0.05$; Figure 4b). Stratifying by historical CO_2 threshold, subjects in each subgroup responded similarly ($P = 0.25$)—but the subgroups themselves responded differently ($P < 0.05$)—to increasing CO_2 concentrations.

ΔpH was also measurable at threshold for six of eight subjects, including three in the low-threshold group and three in the high-threshold group. Among these six subjects, there was a significant positive correlation between log-transformed [CO_2 threshold] and mean ΔpH at threshold (Figure 5; $P < 0.05$). Stated differently, individuals who were 'less sensitive' to CO_2 (i.e. who had higher CO_2 detection thresholds) were responding to greater pH changes at threshold than were 'more sensitive' subjects (those with lower CO_2 detection thresholds).

Latency to peak pH deviation significantly decreased with either applied [CO_2] or ΔpH, with the strongest relationship

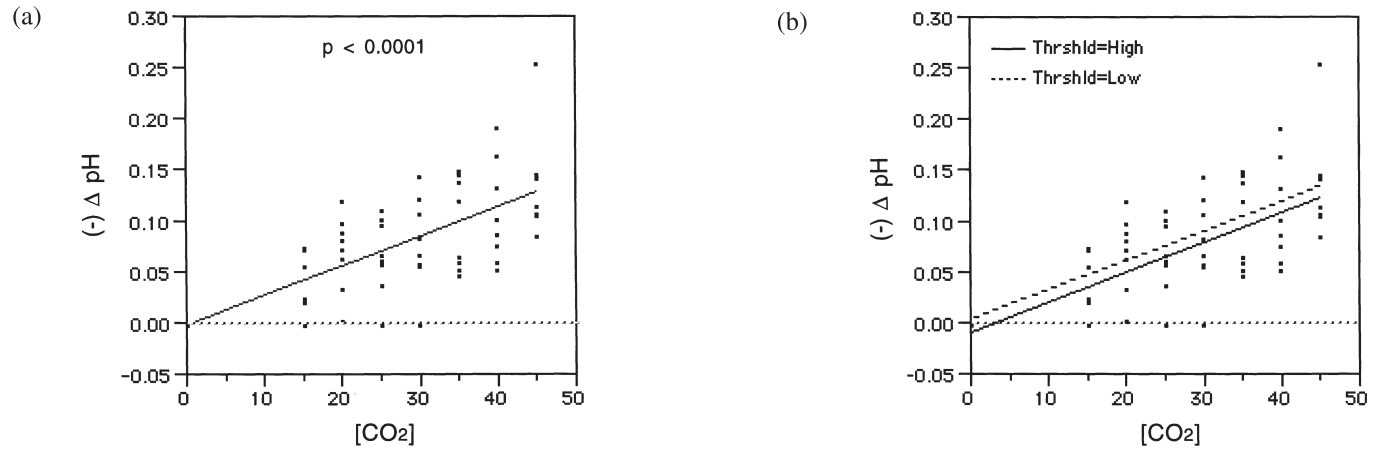

Figure 2 **(a)** Individual mean values of ΔpH (inverted scale) as a function of applied [CO_2], all subjects combined ($P < 0.0001$). **(b)** Stratified by historical CO2 threshold, there was no significant difference in regression lines between low-threshold and high-threshold subjects. Each dot represents the mean of five observations, within individual and stimulus series.

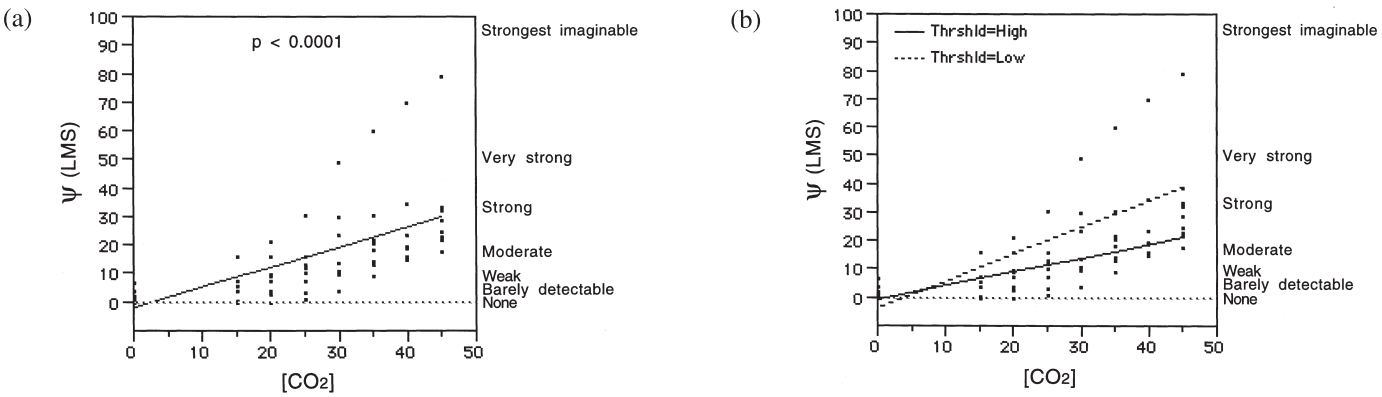

Figure 3 **(a)** Individual mean values of ψ (LMS scale) as a function of applied [CO_2], all subjects combined ($P < 0.0001$). **(b)** Stratified by historical CO_2 threshold, low-threshold subjects had a significantly steeper regression line than did high-threshold subjects ($P < 0.01$). Each dot represents the mean of five observations, within individual and stimulus series.

being to the latter variable ($P < 0.0001$; Figure 6). The shortening of latency with increasing stimulus concentration is a consistent effect across subjects ($P < 0.01$).

Discussion

CO_2 stimulation has widespread application in nasal chemosensory studies, including those involving detection thresholds (Stevens *et al.*, 1982; Anton *et al.*, 1992; Shusterman *et al.*, 2001), thresholds for transient respiratory disruption (Cometto-Muniz and Cain, 1982; Dunn *et al.*, 1982; Stevens *et al.*, 1982), cortical evoked responses (Barz *et al.*, 1997; Hummel and Kobal, 1992; Hummel *et al.*, 1998), magnetoencephalography (Huttunen *et al.*, 1986; Hari *et al.*, 1997) and mucosal electrophysiologic responses (Kobal, 1985; Thurauf *et al.*, 1991, 1993; Hummel *et al.*, 1996). Notwithstanding this agent's widespread use in this context, knowledge of its mechanism of action is largely indirect. The belief that CO_2 acts by hydration to H_2CO_3, dissociation to H^+, and stimulation of acid-sensitive ion channels is based primarily on studies involving either: (i) inhibition of carbonic anhydrase, the enzyme facilitating CO_2's hyration/dissociation (Alimohammadi and Silver, 2001; Dessirier *et al.*, 2000); or (ii) pharmacological inhibition of a subpopulation of acid-sensing receptors (in particular, VR1; Alimohammadi and Silver, 2002).

We directly examined this model of stimulus dynamics by applying a novel mucosal monitoring technique to CO_2-induced trigeminal irritation. A small-diameter pH probe introduced into the floor of one nostril during CO_2 stimulation yielded real-time pH measurements during psychophysical testing, measurements which then could be compared with both stimulus [CO_2] and response (threshold and suprathreshold scaling) data (hypothesis 1). Averaging across subjects, a significant dose–response relationship was

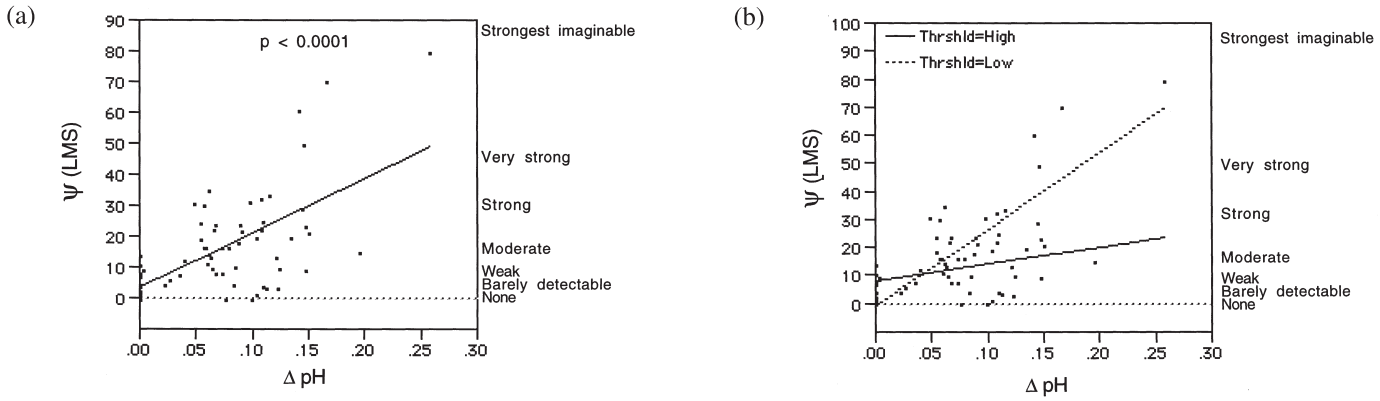

Figure 4 **(a)** Relationship between individual mean ΔpH and ψ, averaged by stimulus level, all subjects combined ($P < 0.001$). **(b)** Stratified by historical CO_2 threshold, low-threshold subjects had a significantly steeper regression line than did high-threshold subjects ($P < 0.05$). Each dot represents the mean of five observations, within individual and stimulus series.

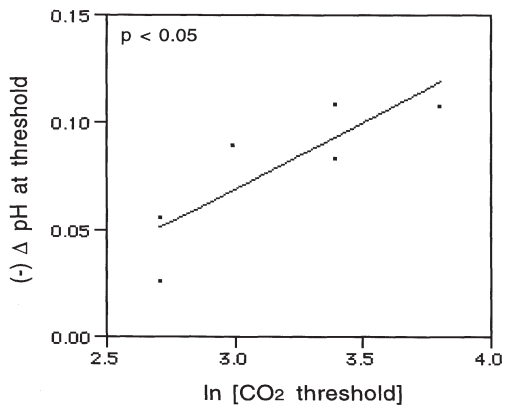

Figure 5 Relationship between [log-transformed] CO_2 detection threshold and ΔpH at threshold for six of eight subjects in which measurable pH fluctuations were observed at that stimulus step. ΔpH at threshold increased significantly with increasing [log-transformed] CO_2 detection threshold ($P < 0.05$).

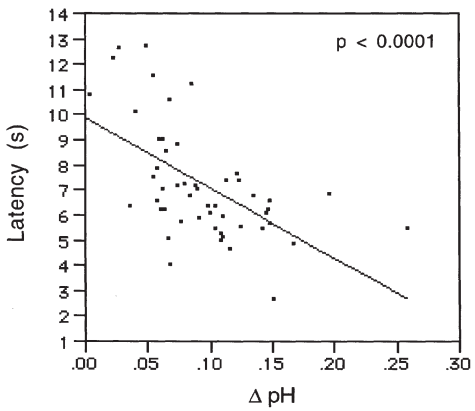

Figure 6 Relationship of latency to peak pH deviation to ΔpH on a trial-to-trial basis, all subjects combined. There is a significant negative correlation ($P < 0.0001$).

observed between applied stimulus concentration [CO_2] and both ψ and ΔpH (hypothesis 2). In addition, mean ψ and ΔpH were significantly interrelated (hypothesis 3). In a stratified analysis, historically high- and low-CO_2 threshold groups differed with regard to the relationship between ψ and both [CO_2] and Δ, but not the relationship between [CO_2] and ΔpH, with low-threshold ('sensitive') individuals showing steeper dose–response curves (hypotheses 4 + 5). For the six of eight subjects in whom phasic pH changes were apparent at their CO_2 detection threshold step, ΔpH was greater at threshold in those having higher [log-transformed] CO_2 thresholds (hypothesis 6). Finally, latency to peak pH deviation decreased with increasing stimulus concentration, possibly reflecting buffering kinetics in nasal mucus.

Our results show that a widely used psychophysical model can be broken into compartments (Figure 7). Measurement of ΔpH in nasal mucus accesses an intermediate step in CO_2 stimulus dynamics. Despite the robust associations observed, however, further dissection of the process may be instructive. Specifically, it is unclear to what degree pH changes in nasal mucus represent parallel pH changes within the epithelial or subepithelial cell layers (i.e. in the vicinity of trigeminal nerve endings). Animal studies utilizing pH microelectrodes and/or supravital dyes could potentially provide this information.

An important aspect of our CO_2 nasal chemosensory work concerns inter-individual variability. Numerous studies have documented differences in CO_2 chemosensitivity by age, gender, smoking status, rhinitis status, and olfactory function (for review, see Shusterman, 2002). The biological basis for these differences is speculative at this time, but possibilities include: (i) interindividual differences in CO_2 activation (i.e. carbonic anhydrase enzymatic activity); (ii) differences in mucus buffering; and (iii) differences in nociceptor sensitivity. Based on the dose–response relationships

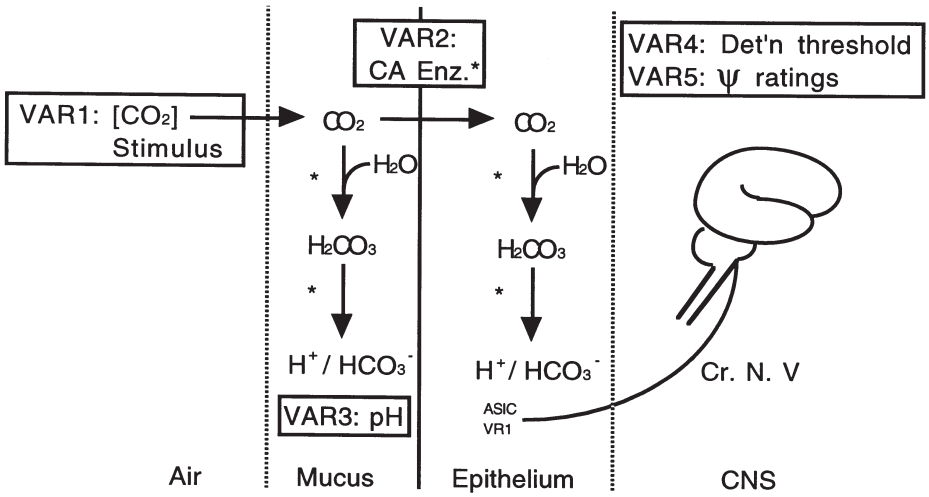

Figure 7 Theoretical framework for studies relating applied stimulus concentration [CO2], ΔpH, CO_2 detection threshold, and suprathreshold rating of sensory irritation ψ. Other abbreviations: CA, carbonic anhydrase enzyme activity; ASIC, acid-sensitive ion channel; VR1, vanilloid (capsaicin/H+/heat) receptor (also referred to as TRPV1); Cr. N. V, fifth (trigeminal) cranial nerve.

observed, our data suggest that historically high- and low-threshold individuals differ in their threshold and suprathreshold response to a given ΔpH excursion, rather than in their activation or buffering of CO_2 pulses. This implies that intrinsic differences in neural sensitivity to H^+ may play a role in chemesthetic variability. Notwithstanding this finding, complementary studies in our laboratory are currently addressing stimulus activation by documenting inter-individual differences in carbonic anhydrase gene expression in the human nasal mucosa (Tarun *et al.*, 2003).

To our knowledge, these data provide the first direct evidence that CO_2-induced nasal sensory irritation reflects short-term decreases in nasal mucosal pH. This technique provides an initial view of pH dynamics with acidic air pollutants and, in theory, could be applied to basic compounds (e.g. ammonia vapor, sodium carbonate dust) as well. Unexplored aspects of the CO_2/ΔpH/ψ relationship include potential stimulus duration effects and further exploration of anatomical subcompartments (see above). However, the current study provides an important first step in understanding mucosal stimulus dynamics involving rapid pH changes.

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References

Alimohammadi, **H.** and **Silver, W.L.** (2001) *The effects of acetazolamide on trigeminal sensitivity to nicotine and carbon dioxide*. Chem Senses, 26, 1046 [abstract].

Alimohammadi, **H.** and **Silver, W.L.** (2002) *In vivo effects of capsazepine on trigeminal nerve sensitivity to carbon dioxide and nicotine [abstract].* Proc. 2002 Annual Meeting of the Association for Chemoreception Sciences, Sarasota, FL.

Anton, **F.**, **Euchner, I.** and **Handwerker, H.O.** (1992) *Psychophysical examination of pain induced by defined CO2 pulses applied to the nasal mucosa*. Pain, 49, 53–60 .

Barz, **S.**, **Hummel, T., Pauli, E., Majer, M., Lang, C.J.** and **Kobal, G.** (1997) *Chemosensory event-related potentials in response to trigeminal and olfactory stimulation in idiopathic Parkinson's disease.* Neurology, 49, 1424–1431 .

Bryant, **B.P.** (2000) *The roles of carbonic anhydrase in gustation, olfaction and chemical irritation.* In Chegwidden, W.R., Carter, N.D. and Edwards, Y.H. (eds), The Carbonic Anhydrases: New Horizons. Birkhauser Verlag, Basel, pp. 365–374.

Cavaliere, **F.**, **Masieri, S., Proietti, R.** and **Magalini, S.I.** (1988) *pH and electrolytes in nasal secretum of intensive care unit patients.* Resuscitation, 16, 133–137.

Cometto-Muniz, **J.E.** and **Cain, W.S.** (1982) *Perception of nasal pungency in smokers and nonsmokers*. Physiol Behav 29, 727–731.

Dessirier, **J.M.**, **Simons, C.T., Carstens, M.I., O'Mahony, M.** and **Carstens, E.** (2000) *Psychophysical and neurobiological evidence that the oral sensation elicited by carbonated water is of chemogenic origin*. Chem Senses, 25, 277–284.

Dunn, **J.D.**, **Cometto-Muniz, J.E.** and **Cain, W.S.** (1982) *Nasal reflexes: Reduced sensitivity to CO_2 irritation in cigarette smokers*. J. Appl. Toxicol., 2, 176–178.

England, **R.J.**, **Homer, J.J., Knight, L.C.** and **Ell, S.R.** (1999) *Nasal pH measurement: a reliable and repeatable parameter.* Clin. Otolaryngol., 24, 67–68.

Gescheider, **G.A.** (1985) Psychophysics: Method, Theory, and Application, 2nd edn. Hillsdale, NJ, Lawrence Erlbaum Associates, pp. 7–11.

Green, **B.G.**, **Dalton, P., Cowart, B., Shaffer, G., Rankin, K.** and **Higgins, J.** (1996) *Evaluating the 'Labeled Magnitude Scale' for measuring sensations of taste and smell.* Chem. Senses, 21, 323–334.

Hari, **R.**, **Portin, K., Kettenmann, B., Jousmaki, V.** and Kobal, G. (1997) *Right-hemisphere preponderance of responses to painful CO_2 stimulation of the human nasal mucosa*. Pain, 72, 145–151.

Hehar, **S.S.**, **Mason, J.D., Stephen, A.B., Washington, N., Jones, N.S., Jackson, S.J.** and **Bush, D.** (1999) *Twenty-four hour ambulatory nasal pH monitoring.* Clin. Otolaryngol., 24, 24–25.

Hummel, **T.** and **Kobal, G.** (1992) *Differences in human evoked potentials related to olfactory or trigeminal chemosensory activation*. Electroencephalogr. Clin. Neurophysiol., 84, 84–89.

Hummel, **T.**, **Schiessl, C., Wendler, J.** and **Kobal, G.** (1996) *Peripheral electrophysiological responses decrease in response to repetitive painful stimulation of the human nasal mucosa*. Neurosci. Lett. 212, 37–40 .

Hummel, **T.**, **Barz, S., Pauli, E.** and **Kobal, G.** (1998) *Chemosensory event-related potentials change with age.* Electroencephalogr. Clin. Neurophysiol., 108, 208–217 .

Huttunen, **J.**, **Kobal, G., Kaukoranta, E.** and **Hari, R.** (1986) *Cortical responses to painful CO_2 stimulation of nasal mucosa*; *a magnetoencephalographic study in man.* Electroencephalogr. Clin. Neurophysiol., 64, 347–349 .

Kobal, **G.** (1985) *Pain-related electrical potentials of the human nasal mucosa elicited by chemical stimulation*. Pain, 22, 151–163 .

Selimoglu, **M.A.**, **Selimoglu, E.** and **Kurt, A.** (1999) *Nasal mucociliary clearance and nasal and oral pH in patients with insulin-dependent diabetes*. Ear Nose Throat J., 78, 585–588, 590.

Shusterman, **D.** (2002) *Individual factors in nasal chemesthesis*. Chem. Senses, 27, 551–564.

Shusterman, **D.J.** and **Balmes, J.R.** (1997) *A comparison of two methods for determining nasal irritant sensitivity*. Am. J. Rhinol., 11, 371–378.

Shusterman, **D.**, **Murphy, M.A.** and **Balmes, J.** (2001) *The influence of sex, allergic rhinitis, and test system on nasal sensitivity to airborne irritants: a pilot study*. Environ. Health Perspect., 109, 15–19.

Stevens, **J.C.**, **Plantinga, A.** and **Cain, W.S.** (1982) *Reduction of odor and nasal pungency associated with aging.* Neurobiol. Aging, 3, 125–132.

Thurauf, **N.**, **Friedel, I., Hummel, C.** and **Kobal, G.** (1991) *The mucosal potential elicited by noxious chemical stimuli with CO2 in rats: is it a peripheral nociceptive event?* Neurosci Lett 128, 297–300.

Thurauf, **N.**, **Hummel, T., Kettenmann, B.** and **Kobal, G.** (1993) *Nociceptive and reflexive responses recorded from the human nasal mucosa.* Brain Res., 629, 293–299.

Tarun A., **Bryant B., Zhai, W., Solomon,C.** and **Shusterman, D.** (2003) *Gene expression for carbonic acid anhydrase isoenzymes in human nasal mucisa*. Chem. Senses, 28, 000–000.

Washington, **N.**, **Steele, R.J., Jackson, S.J., Bush, D., Mason, J., Gill, D.A., Pitt, K.** and **Rawlins, D.A.** (2000) *Determination of baseline human nasal pH and the effect of intranasally administered buffers.* Int. J. Pharmacol., 198, 139–146.

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