

Published in final edited form as:

Life Sci. 2010 July 3; 87(1-2): 36–41. doi:10.1016/j.lfs.2010.05.013.

Trigeminal antihyperalgesic effect of intranasal carbon dioxide

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Abstract

Aims—Clinical studies demonstrate attenuation of trigeminal-related pain states such as migraine by intranasal CO₂ application. This study investigated the underlying mechanisms of this observation and its potential use to reverse trigeminal pain and hypersensitivity.

Main methods—We used a behavioral rat model of capsaicin-induced trigeminal thermal hyperalgesia, intranasal CO₂ application and several pharmacologic agents such as carbonic anhydrase, acid-sensing ion channels (ASICs), and TRPV1 blocker as well as acidic buffer solutions to investigate and mimic the underlying mechanism.

Key findings—Intranasal CO₂ application produced a robust dose-dependent antihyperalgesic effect in rats that lasted at least one hour. Blockade of nasal carbonic anhydrase with a dorzolamide solution (Trusopt® ophthalmic solution) showed only a non-significant decrease of the antihyperalgesic effect of intranasal CO₂ application. Pharmacologic blockade of ASICs or TRPV₁ receptor significantly attenuated the antihyperalgesic effect of CO₂ application. The effect of intranasal CO₂ application could be mimicked by application of pH4, but not pH 5, buffer solution to the nasal mucosa. As with CO₂ application, the antihyperalgesic effect of intranasal pH4 buffer was blocked by nasal application of antagonists to ASICs and TRPV₁ receptors.

Significance—Our results indicate that intranasal CO₂ application results in a subsequent attenuation of trigeminal nociception, mediated by protonic activation of TRPV₁ and ASIC channels. A potential central mechanism for this attenuation is discussed. The antihyperalgesic effects of intranasal CO₂ application might be useful for the treatment of trigeminal pain states.

Keywords

trigeminal; pain; hyperalgesia; hypersensitivity; capsaicin; TRPV₁; ASIC; carbon dioxide

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Competing interests

Capnia, Inc., provided funding for this project. Also, Dr. Yeomans is on the Scientific Advisory Board of Capnia.

Introduction

Trigeminal nerve-mediated pain has a major impact on human health, and significantly debilitates the functioning of the afflicted (Tölle T et al. 2006; Bigal M et al. 2007). Conditions such as migraine headache, trigeminal neuralgia, non-migraine headache and dental pain reduce well-being and negatively affect productivity and the economy. Unfortunately, many pharmacological approaches to alleviate trigeminal pain are rife with harmful side effects and/or risks of addiction, necessitating a continued search for other methods of pain relief.

One treatment for trigeminal pain under development is the nasal application of carbon dioxide (CO₂). There is evidence that CO₂ can decrease the release of pain-related neurotransmitters from cultured sensory neurons (Vause C et al. 2007). In addition, clinical observations have found intranasal CO₂ to alleviate headache by an unknown mechanism (Spierings EL 2005). On the other hand, intranasal CO₂ is known to produce discharges in rat trigeminal afferent (Thurauf N et al. 1991) as well as medullary neurons (Anton F et al. 1991) and a stinging nasal pain sensation in human subjects (Hummel T et al. 2000; Hummel T et al. 2003), which has led to its use as a noxious stimulus in psychophysical pain studies (Hummel T et al. 2003).

Previous studies have observed the production of a widespread analgesic effect by activation of nociceptive afferents (Cui JG et al. 1997; Sandkuhler J 2000). For example, in a recent study using a herpes-mediated GABA_b receptor knockdown, we found that repeated activation of A δ -thermonociceptors in the sciatic nerve of rats produced hypoalgesia for responses mediated by activation of saphenous nociceptors, an effect mediated by activation of inhibitory spinal interneurons (Jones TL et al. 2005). Thus, it is possible that the apparent trigeminal antinociceptive effect of nasal CO₂ on migraine headache and other trigeminal pain might be mediated through a similar mechanism of widespread nociceptive inhibition evoked by afferent activation.

Therefore, we investigated whether nasal CO₂ would attenuate nociceptive behavioral responses to stimuli applied to the trigeminally-innervated facial skin of rats.

Methods

Animals

Male Sprague-Dawley rats (200 to 300 g, Charles River) were housed in a 12-h light: 12-h dark environment and provided food and water *ad libitum*. Effort was made to minimize discomfort and to reduce the number of animals used. At the end of each experiment, animals were euthanized by CO₂ asphyxiation. The protocol of this study was approved by the Stanford University Institutional Animal Care and Use Committee.

Heat withdrawal test

Animals were lightly anesthetized with urethane (1000 mg/kg *ip*). It has been shown in previous studies that light urethane anesthesia (800–1000 mg/kg *ip*) does not alter withdrawal latencies to noxious thermal stimuli administered to paws (Yeomans DC et al. 1996; Zachariou V et al. 1997) or areas innervated by the trigeminal nerve (Tzabazis A et al. 2004). The experimenter performing the behavioral tests was blinded to the rat treatment group assignment. Thirty minutes after urethane administration, the animals' faces were shaved closely with a hair clipper without causing damage to the skin and blackened with India ink applied with a single-use cotton swab. All animals were tested for baseline withdrawal latency using a heat lamp set at a C thermociceptor selective intensity of 190 mW/cm² (Yeomans DC et al. 1996; Yeomans DC and Proudfit HK 1996). The mean latency

to response to noxious heat (facial withdrawal or flinching) was calculated as the average of four trials' response time in seconds. Maximum (cut-off) latency was set at 20 s to avoid tissue damage to the face.

Capsaicin sensitization and CO₂ administration

After baseline latencies were obtained, one cheek of the animals was sensitized with two 20 µl applications, administered 20 minutes apart, of 15 mM synthetic capsaicin (Sigma-Aldrich, St. Louis, MO, USA) applied and spread with a pipette tip. Twenty minutes after the second application, the animals were tested again for response latencies to heat. CO₂ delivered from a cylinder containing 100% CO₂ and was administered using an adjustable pressure reducer via a single-use 14G intravenous catheter (BD Insyte™ Autoguard™, Becton, Dickinson Infusion Therapy systems Inc., Sandy, USA) inserted approximately 1 mm into one nare. The rats were then tested immediately, and at 30 and 60 minutes after CO₂ administration (n = 9). Three groups of animals were treated with CO₂ at a flow rate of 0.8 l/min for 20 s, 0.8l/min for 40 s, and 0.4 l/min for 40 s (n = 9 per group), respectively. For control groups, some rats received compressed air at a flow rate of 0.8 l/min for 40 s (n = 9); another group was treated with saline applied to the skin and spread using a pipette tip and received CO₂ at a flow rate of 0.8 l/min for 40 s (n = 9). As a third and fourth control group animals treated with saline but no CO₂ or sensitized with capsaicin without intranasal CO₂ (n=9), respectively, were tested at time points to match time points of the groups mentioned above. Hind paw withdrawal latencies were tested in two additional groups after capsaicin sensitization of the cheek and with or without intranasal administration of CO₂ at a flow rate of 0.8l/min for 40 s.

Pharmacological experiments

To investigate potential underlying mechanisms of CO₂ antihyperalgesia, capsaicin sensitized animals were pretreated with 50 µl of a 55.4 mM solution of the clinically available carbonic anhydrase blocker dorzolamide (Trusopt®, MSD Inc, White House Station, NJ, USA). Fifteen minutes later, rats were administered CO₂ at a flow rate of 0.8 l/min for 40 seconds (n = 6). Dorzolamide has been used to block carbonic anhydrase in cultured retinal neural cells (Knier EM et al. 2006) as well as in mice after topical application on the retina (Akaishi T et al. 2009).

A second group (n = 6) was nasally pretreated with 50 µl of 2 mM solution of the non-specific acid sensing channel (ASICs) blocker amiloride (Sigma-Aldrich, St. Louis, MO, USA) in normal saline applied topically to the nostril. Amiloride has been shown to dose-dependently block ASICs in neurons (Leffler A et al. 2006, Zhang M et al. 2008). Fifteen minutes after applying amiloride, rats were administered CO₂ nasally at a flow rate of 0.8 l/min for 40 seconds (n = 6).

A third group (n = 6) was nasally pretreated with 50 µl of a 5 mM solution of the TRPV₁ channel blocker capsazepine (Sigma-Aldrich, St. Louis, MO, USA) in 10% tween 20 (Bio-Rad, Hercules, CA, USA), 10% ethanol (EMD chemicals, USA) and 80% normal saline. Capsazepine has been shown to be highly selective in blocking TRPV₁ with low affinity for other channels (Seabrook GR et al. 2002, Leffler A et. al. 2006). Fifteen minutes after applying capsazepine, rats were nasally administered CO₂ at a flow rate of 0.8 l/min for 40 seconds (n = 6). Appropriate control groups (n=6), *i. e.* treated with the amiloride and capsazepine vehicle, respectively, were also tested. Since we used a commercially available formulation of dorzolamide in an a proprietary aqueous solution with inactive ingredients such as hydroxyethyl cellulose, mannitol, sodium citrate dihydrate, and sodium hydroxide in unknown amounts (Trusopt®), we could not include a group treated with the appropriate dorzolamide vehicle. A fourth group (n = 6) was nasally pretreated with 50 µl of amiloride

and 50 μ l of capsazepine, then administered CO₂ at a flow rate of 0.8 l/min for 40 seconds (n = 6). To investigate the possibility that CO₂ induced antihyperalgesia was mediated by acidification of the nasal mucosa, additional groups (n = 6 each) were administered four 6 μ l applications of pH 4 and pH 5 buffer, respectively, per nostril, 2 minutes apart. This application method was found to be the most effective application method in preliminary studies. Effects of cheek withdrawal latencies were then tested immediately, and at 30 and 60 minutes after the fourth buffer administration. As a control, other groups (n = 6 each) were pretreated with amiloride or capsazepine, respectively, as described above before intranasal administration of pH 4 buffer and tested in identical manner.

Statistics

Data is presented as mean \pm SEM. Behavioral data was analyzed for significance by two-way ANOVA with repeated measures followed by a post-hoc Bonferroni analysis. P-values lower than 0.05 were considered significant. All statistical analysis were performed using GraphPad Prism version 5.0 for Mac OS X (GraphPad Software, San Diego California USA, <http://www.graphpad.com>).

Results

Effect of CO₂ and air on capsaicin sensitized cheek

After sensitization of facial skin of rats with topical capsaicin, facial withdrawal response latencies evoked by C fiber selective radiant heat dropped from 10.2 \pm 1.1 s to 5.3 \pm 0.8 s (Fig. 1). The analysis of variance indicated that withdrawal latency was significantly affected by treatment ($F_{0.05,4,16}=10.96$, $F_{crit}=3.01$) and by timepoint ($F_{0.05,4,16}=14.09$, $F_{crit}=3.01$). Lightly anesthetized rats tolerated nasal CO₂ administration well. Slight moving of the head was observed when placing the intravenous catheter into the nare, but usually stopped within a few seconds. CO₂ administration was only started when the animals were lying still.

The group that received CO₂ at 0.8 l/min for 40 s exhibited a withdrawal latency of 13.1 \pm 1.8 s immediately following treatment indicating an antihyperalgesic effect when compared to the post-capsaicin baseline. Similarly, a group that received CO₂ at 0.8 l/min for the shorter period of 20 s exhibited a withdrawal latency of 11.1 \pm 1.6 s immediately following treatment. *Post hoc* Bonferroni analysis revealed that both of these groups' average latency responses were significantly higher than that of the control group that received no CO₂ treatment and not significantly different from one another. The group of animals that received CO₂ at the lower flow rate of 0.4 l/min for 40 s exhibited a latency withdrawal of 7.2 \pm 0.6 s immediately following treatment, which was not significantly different from capsaicin-treated control. Control animals that received air at a flow rate of 0.8 l/min for 40 s exhibited a heat withdrawal latency of 8.1 \pm 0.6 s for the sensitized cheek immediately following treatment, which was also not significantly different from the "capsaicin only" group.

Duration of CO₂ effect on sensitized cheek

Thirty and 60 minutes after CO₂ application, the group that received CO₂ at 0.8 l/min for 40 s exhibited average response latencies of 11.4 \pm 1.6 s and 10.4 \pm 1.1 s, respectively (Fig. 1), which were both significantly higher than the control capsaicin-sensitized group that did not receive CO₂ treatment. Thirty minutes after CO₂ application, the group that received CO₂ at 0.8 l/min for 20 s exhibited an average response latency of 9.7 \pm 1.7 seconds, which was significantly higher than control. Sixty minutes after treatment this effect (8.8 \pm 1.2 s) was no longer significant, there was however a trend towards an increased withdrawal latency (Fig. 1). At no point were the response latencies of the group that received CO₂ at 0.4 l/min for 40

s or air at 0.8l/min for 40 s significantly higher as compared to the capsaicin-sensitized control group (Fig. 1).

Effect of CO₂ on nonsensitized cheek skin

Withdrawal latencies of the group that received CO₂ (0.8 l/min for 40 s) after topical application of saline to the cheek instead of capsaicin exhibited a small increase in withdrawal latency to 13.8±0.5 s immediately after treatment, which indicated a significant antinociceptive effect when compared to non-sensitized control animals that did not receive CO₂ (p<0.05; Fig. 2). The control group that received no CO₂ exhibited no statistically significant latency changes over the same time period.

Effect of CO₂ on paw withdrawal latencies

Right paw withdrawal latencies to noxious heat stimulation after capsaicin sensitization of the left cheek were decreased to 7.8±0.7 and 7.2±0.6 s in the group compared to the pre-sensitization baseline (8.8±0.4 and 8.9±0.6 s). In the group that received intranasal administration of CO₂ at a flow rate of 0.8l/min for 40 s paw withdrawal latencies increased to 10.3±0.8 s immediately after, 10.9±0.9 s 30 minutes after, and 10.2±1.1 s 60 minutes after CO₂ treatment compared to 7.8±1.1 s, 7.5±1.0 s, and 8.8±0.8 s for the animals that did not receive CO₂ (Fig. 3). The analysis of variance indicated that withdrawal latency was significantly affected by treatment ($F_{0.05,4,16}=8.51$, $F_{crit}=3.01$).

Pharmacology

All animals tested for pharmacology were administered CO₂ at 0.8 l/min for 40 s based on the hypothesis that CO₂ would acidify the nasal mucosa and that protons thus released would activate nasal nociceptors. The analysis of variance indicated that cheek withdrawal latencies were significantly increased by treatment ($F_{0.05,5,20}=11.26$, $F_{crit}=2.71$) and by timepoint ($F_{0.05,4,16}=8.97$, $F_{crit}=3.01$). *Post-hoc* Bonferroni analysis revealed that the average withdrawal response latency of 10.8±0.9 s for animals that received CO₂ treatment following nasal application of the carbonic-anhydrase inhibitor dorzolamide (Trusopt® ophthalmic solution) indicated a non-significant decrease from the antihyperalgesia evidenced in the group treated only with CO₂ (Fig. 3A). Similarly, at 30 and 60 minutes after CO₂ exposure, animals pretreated with dorzolamide showed a non-significant attenuation of the antihyperalgesic effect of CO₂ as indicated by a decrease in post-CO₂ withdrawal latencies compared to animals exposed to CO₂ without dorzolamide pretreatment (Fig. 4A).

Animals that received CO₂ treatment following nasal pretreatment with the mixed ASIC channel blocker amiloride exhibited a response latency of 7.3±0.7 s in the sensitized cheek immediately after CO₂ treatment, which was significantly lower than response latencies of the group that received only CO₂, indicating an attenuation of the antihyperalgesic effect and possible mediation of that effect by ASICs. Thirty and 60 minutes after CO₂ exposure withdrawal latencies were 7.5±1.3 s and 6.8±1.0 s, respectively which were also significantly shorter than those of animals treated with CO₂ without amiloride pretreatment (Fig. 4B). Animals intranasally treated with the appropriate vehicle for amiloride prior to intranasal CO₂ application did not show a significant attenuation of the antihyperalgesic effect (p>0.05, data not shown).

Similarly, rats nasally pretreated with the TRPV₁ blocker capsazepine exhibited response latencies of 8.0±0.6 s in the sensitized cheek after CO₂ treatment, which was significantly lower than the group treated only with CO₂, and remained so at 30 (6.7±0.4 s) and 60 minutes (6.9±0.5 s) after treatment indicating a robust blockade of the antihyperalgesic effect of CO₂ (Fig. 4C). Again, pretreatment with the appropriate vehicle did not lead to an

attenuation of the antihyperalgesic effect observed after intranasal CO₂ at 0.8 l/min for 40 s ($p>0.05$, data not shown).

The group that was pretreated with both amiloride and capsazepine showed significantly shorter withdrawal latencies, when compared to capsaicin-sensitized animals treated with CO₂ alone, immediately, 30, and 60 minutes after CO₂ treatment as well ($p<0.05$, data not shown).

Effect of acidic buffer on withdrawal responses of capsaicin-sensitized cheeks

In an effort to further examine the influence of the acidification of the nasal mucosa by CO₂, a group of animals that received intranasal pH 4 buffer but not those that received pH 5 buffer treatment exhibited a significantly elevated average cheek withdrawal latency (12.0 ± 0.6 s) in sensitized rats immediately after treatment when compared to controls (Fig. 4). Analysis of variance indicated that withdrawal latency was significantly affected by treatment ($F_{0.05,5,20}=11.85$, $F_{crit}=2.71$) and by timepoint ($F_{0.05,4,16}=21.28$, $F_{crit}=3.01$). *Post-hoc* Bonferroni analysis demonstrated a significant attenuation of the capsaicin-induced hyperalgesia by intranasal pH 4 buffer, but no significant difference from the average response latency of the group that received CO₂ (0.8 l/min, 40 s). At thirty and 60 minutes after intranasal application of pH 4 buffer, no antihyperalgesic effect observed, indicating a relatively short duration of effect.

Animals that received pretreatment with amiloride or capsazepine prior to buffer application exhibited significantly lower withdrawal latencies immediately after intranasal pH4 buffer administration as compared to animals that received pH4 buffer alone. Thirty and 60 minutes after buffer administration, there was no significant difference between groups (Fig. 5).

Discussion

Our results indicate that intranasal administration of CO₂ produces a dose-dependent antinociceptive/antihyperalgesic effect on trigeminal nociception. Forty seconds of CO₂ at a flow rate of 0.8l/min produced a substantial attenuation of trigeminal nociception in capsaicin-sensitized rats for at least 60 minutes. In addition, animals that were not sensitized with capsaicin prior to nasal application of CO₂ showed a significant increase in withdrawal latencies to a heat stimulus indicating, a slight antinociceptive effect. We hypothesized that the mechanism underlying these effects involves nasal mucosal carbonic anhydrase catalysis of CO₂ to form carbonate and protons. Hypothetically, the resulting free protons would activate acid sensing ion channels (ASICs) as well as TRPV₁ channels on mucosal trigeminal nociceptor terminals, resulting in afferent activation. This afferent activity, upon reaching the CNS, would then induce a wide-spread inhibition of further incoming trigeminal nociceptive activity, probably through activation of inhibitory interneurons (Hummel T et al. 2000; Jones TL et al. 2005) or by activation of trans-segmental inhibitory control circuitry (Bouhassira D et al. 1995). Some components of this hypothesis were tested using behavioral pharmacological methods.

Forty seconds of CO₂ exposure of the nasal mucosa at a flow rate of 0.8 l/min was found to produce a clear facial antihyperalgesic effect. In evidence that this effect was mediated by catalysis of CO₂ to protons and bicarbonate, amiloride, an ASIC inhibitor, was found to significantly decrease the antihyperalgesic effect of CO₂. Amiloride is a diuretic that has been shown to block ASIC channels (Ferreira J et al. 1999), and to suppress ASIC mediated responses in patch clamp experiments (Dube GR et al. 2005) as well as in vivo rodent pain models (Ferreira J et al. 1999; Dube GR et al. 2005).

Pretreatment of the nasal mucosa with the TRPV₁ antagonist capsazepine blocked the antihyperalgesia produced by CO₂ application indicating the involvement of TRPV₁ channels. Dorzolamide solution, a clinically available carbonic anhydrase inhibitor formulated for ophthalmic mucosa (Trusopt®), demonstrated only a non-significant trend in attenuating the CO₂ mediated antihyperalgesia at the tested concentration despite the fact that carbonic anhydrase is expressed in 79% of TRPV₁-positive trigeminal neurons (Tanimoto T et al. 2005). The non-significance of this finding may be due to a lack of alternative concentrations of the Trusopt® product or an attenuation of the anti-hyperalgesic effect by one of the numerous excipients of the dorzolamide formulation such as sodium hydroxide, which is a strong base. However, since other carbonic anhydrase inhibitors such as acetazolamide are insoluble in aqueous solution, we were limited to the Trusopt® preparation.

Lastly, to provide additional evidence that it was, in fact mucosal acidification that produced the antihyperalgesia, pH 4 buffer was administered to the nose of animals sensitized with capsaicin and was found to have a significant antihyperalgesic effect of short duration. Notably, in animals not sensitized with capsaicin, pH 4 buffer was not found to have an effect, again suggesting proton signaling in the induction of CO₂ antihyperalgesia. One possible explanation for the shorter-termed effect of the nasal pH 4 buffer solution as compared to the intranasal CO₂ administration is the different temporal and spatial distribution of an aqueous solution when compared to CO₂ gas. In humans, it has been shown that low-viscosity solutions were swallowed rapidly after nasal administration (Frasnelli J et al. 2005) thus decreasing the time of interaction with the nasal mucosa. On the other hand, CO₂ gas molecules would readily pass through the mucosa. One might also argue that an intranasally administered fluid will not cover the same surface area of the nasal mucosa as compared to an intranasal gas insufflation, thus interacting with fewer afferent receptors. This is consistent with our our observation of a shorter-termed antinociceptive effect after administrating 0.8l/min CO₂ for 20s as compared to the rats that received 0.8l/min CO₂ for 40 s.

Vause et al. (Vause C et al. 2007) have recently shown that treatment of cultured trigeminal neurons with CO₂ under isohydric conditions inhibits sensory nerve activation as induced by capsaicin or KCl, and leads to a subsequent decrease in neuropeptide, i. e. CGRP, release. They proposed an involvement of inhibition of calcium channels. However, Vause et al. also observed a significant increase in CGRP release when trigeminal ganglion neurons were cultured in acidic media and after CO₂ exposure of cell cultures, which also leads to a drop in culture medium pH. These findings support our observation that intranasal CO₂ leads to activation of mucosal primary afferents by protons liberated by a CO₂ induced drop in nasal pH.

Consistent with our rodent findings, Spierings E. L. (Spierings EL 2005; Spierings ELH 2005) presented a randomized, double blind, placebo-controlled, parallel-group study where he investigated the effects of non-inhaled, intranasal CO₂ on migraine headache. In the CO₂ group 29.8% of patients were pain-free at 2 hours having treated migraine headaches of mild, moderate, or severe intensity, whereas only 9.2% of patients in the placebo group were pain-free. Since there were no clinically significant systemic and only minor and short-lived adverse effects observed, it was concluded that non-inhaled, intranasal CO₂ is a well-tolerated and safe alternative for the abortive treatment of migraine headaches.

To our knowledge, there are no previous studies that demonstrated antinociceptive/antihyperalgesic effects of intranasal CO₂ application in a behavioral animal model. Thuerauf et al. investigated mucosal potentials elicited by CO₂ stimuli in rats (Thuerauf N et al. 1991) observing a reduction in amplitudes of negative mucosal potentials after repetitive

application of high-concentration (>90% v/v CO₂) stimuli. Hummel et al. (Hummel T et al. 2003) reported that a continuous stimulus of CO₂ applied to human subjects results in a decrease in pain ratings, sometimes even to non-painful levels as opposed to CO₂ stimuli of a length of up to 1600 ms, which induce robust pain sensations. These observations support the present findings of an antinociceptive/antihyperalgesic effect of nasal CO₂ application, since the 0.8 l/min flow rate applied in this study should lead to a high CO₂ concentration in the nasal cavity. In addition, exposure length for the antinociceptive/antihyperalgesic effect in the present study was 40 s compared to 1.2 s and 0.2 to 1.6 s in the studies of Thuerauf et al. and Hummel et al., respectively.

We have previously demonstrated that tonic activation of sciatic A δ afferents attenuates nociceptive responses mediated by the activation of saphenous C-afferents (Jones TL et al. 2005), implying activation of a trans-segmental inhibitory process. This effect could be counteracted by intrathecal administration of a GABA_B receptor antagonist or by selective silencing of the GABA_B receptor gene in sensory neurons using an HSV vector, demonstrating at least partial mediation by presynaptic GABA_B receptors on nociceptors. Similarly, Cui et al. (Cui JG et al. 1997) and Sandkuhler (Sandkuhler J 2000) have shown that low frequency tonic activation of A δ afferents leads to a centrally mediated “long term depression” of subsequent nociceptive input, i.e. activity of one type of afferents modulates the nociceptive input of other afferents. More recently, Lapirot et al. has shown that facial grooming time after formalin injection into the vibrissa of rats can be suppressed by a conditioning noxious stimulus (formalin injection into the hindpaw, Lapirot O et al 2009). This phenomenon is known as diffuse noxious inhibitory controls (DNIC) and comprises a pathway that is activated when two concomitant painful stimuli are applied at the same time (van Wijk G et al. 2010). Our observation of increased paw withdrawal latencies after capsaicin-sensitization and intranasal CO₂ insufflation suggest that similar central mechanisms are critical in mediating the antinociceptive/antihyperalgesic effect of nasal CO₂. Such potential mechanisms are currently under study in our laboratory.

In summary, the present study suggests a possible mechanism underlying the results of the clinical observations of analgesic effects of nasal CO₂ in patients with migraine. Our results provide evidence for a centrally-mediated antinociceptive/antihyperalgesic effect of intranasal CO₂ application mediated by activation of afferents in the nasal mucosa by protons via ASICs and TRPV₁ channels (Spierings EL 2005). Furthermore, these studies suggest that nasal CO₂ may be useful for the treatment of somatic trigeminal pain states. Considering this and taking into account the clinical effects reported by Spierings (Spierings EL 2005; Spierings ELH 2005), it seems likely that the observed antihyperalgesic effect after intranasal CO₂ administration affects all types of pain mediated by the trigeminal system, and possibly non-trigeminal pain. However, additional investigations are necessary to further elucidate the underlying mechanisms and the potential of this therapeutic approach in humans.

Conclusions

Our results indicate that intranasal CO₂ application might be a useful treatment option for trigeminal pain states. These effects are likely mediated by protonic activation of TRPV₁ and ASIC channels on afferent neurons innervating the nasal mucosa.

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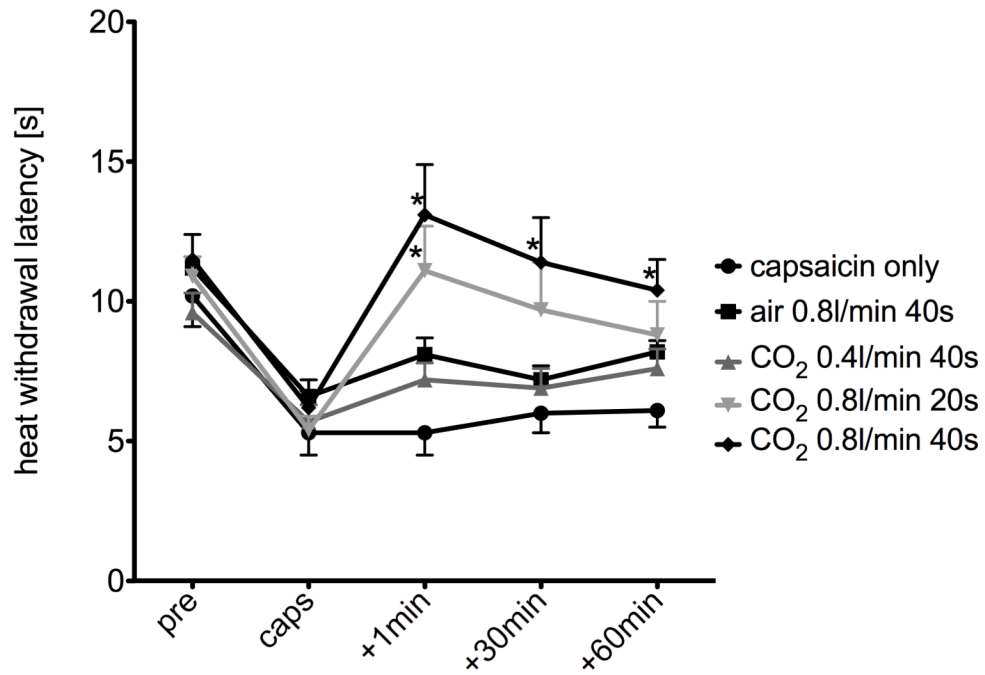


Fig. 1. Effect of intranasal CO₂ on capsaicin-induced heat sensitization: After topical capsaicin application, there was a robust decrease in withdrawal latencies to a heat stimulus applied to the cheek (circles). Air applied with a flowrate of 0.8 l/min for 40 s (squares) and CO₂ with a flowrate of 0.4 l/min for 40 s (dark grey triangles), respectively, did not show significant antihyperalgesic effects. CO₂ with a flowrate of 0.8 l/min for 20 s (light grey triangles) exhibited a significant ($p < 0.05$, ANOVA) antihyperalgesic effect immediately after application, which decreased with time, whereas CO₂ with a flowrate of 0.8 l/min for 40 s (rhombs) demonstrated a significant ($p < 0.05$, ANOVA) antihyperalgesic effect for every observed time point after application. * indicates statistical significance as compared to “capsaicin only” (two way-ANOVA, $p < 0.05$).

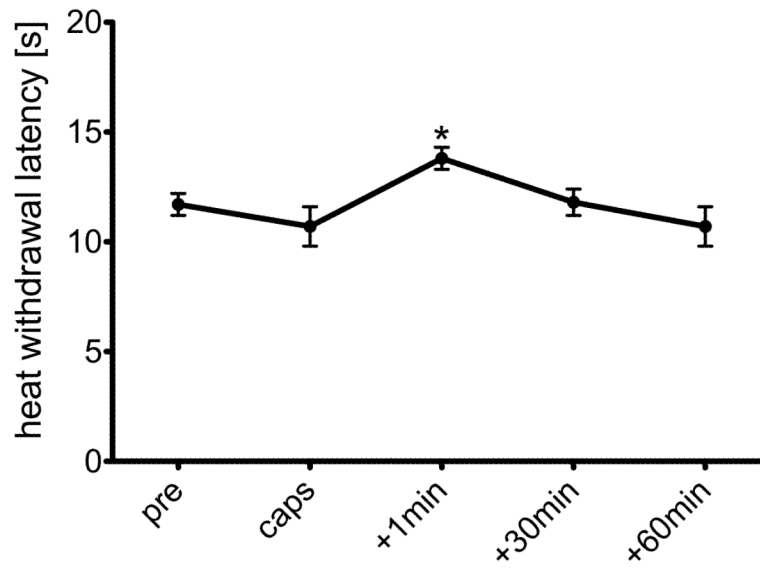


Fig. 2. Effect of intranasal CO₂ insufflation (0.8l/min for 40 s) on heat withdrawal latencies on capsaicin vehicle treated (non sensitized) cheeks. Heat withdrawal latency one minute after CO₂ insufflation was significantly different from baseline indicating a short-termed antinociceptive effect of CO₂ in non-sensitized skin (*: p<0.05, t-test)

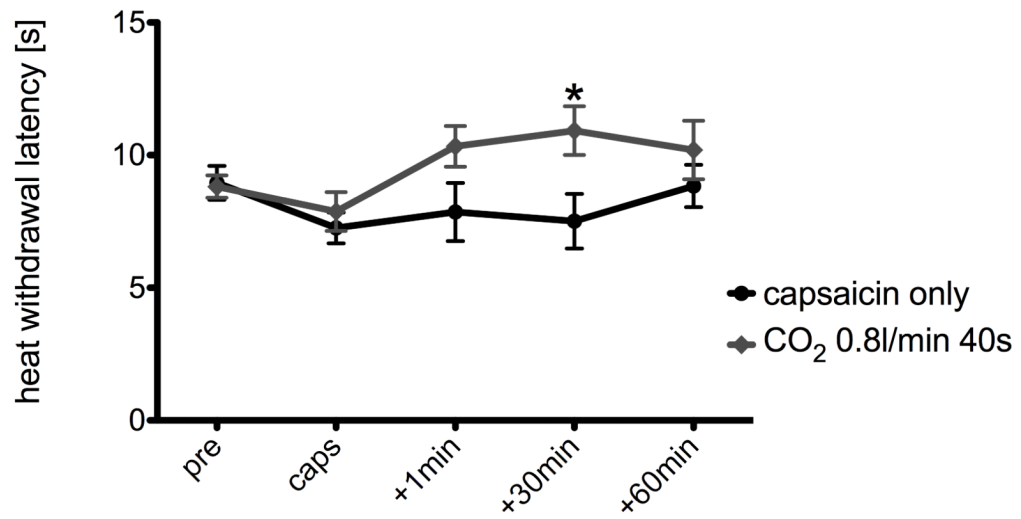


Fig. 3. Effect of intranasal CO₂ insufflation (0.8l/min for 40 s) on right hind paw withdrawal latencies after capsaicin sensitization of the left cheek. * indicates statistical significance as compared to “capsaicin only” (two way-ANOVA, $p < 0.05$)

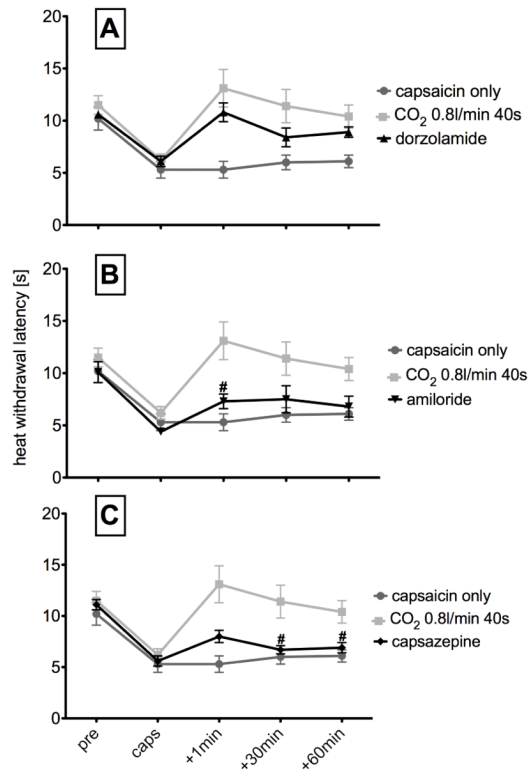


Fig. 4.

Effects of drugs on CO₂ induced antihyperalgesic effect: A) Application of the carbonic anhydrase blocker dorzolamide before intranasal administration of CO₂ with a flowrate of 0.8 l/min for 40 s non-significantly ($p > 0.05$, ANOVA) blocked the CO₂ induced antihyperalgesia as compared to CO₂ application without pretreatment. B) Application of an the acid-sensing ion channel blocker amiloride before intranasal administration of CO₂ with a flow rate of 0.8 l/min for 40 s significantly ($p < 0.05$, ANOVA) blocked the CO₂ induced antihyperalgesic effect immediately after application. C) Application of the TRPV₁ blocker capsazepine before intranasal administration of CO₂ with a flowrate of 0.8 l/min for 40 s also significantly ($p < 0.05$, ANOVA) blocked the CO₂ induced antihyperalgesic effect 30 and 60 minutes after application of CO₂. # indicates statistical significance as compared to “CO₂ 0.8 l/min 40s” (two way-ANOVA, $p < 0.05$).

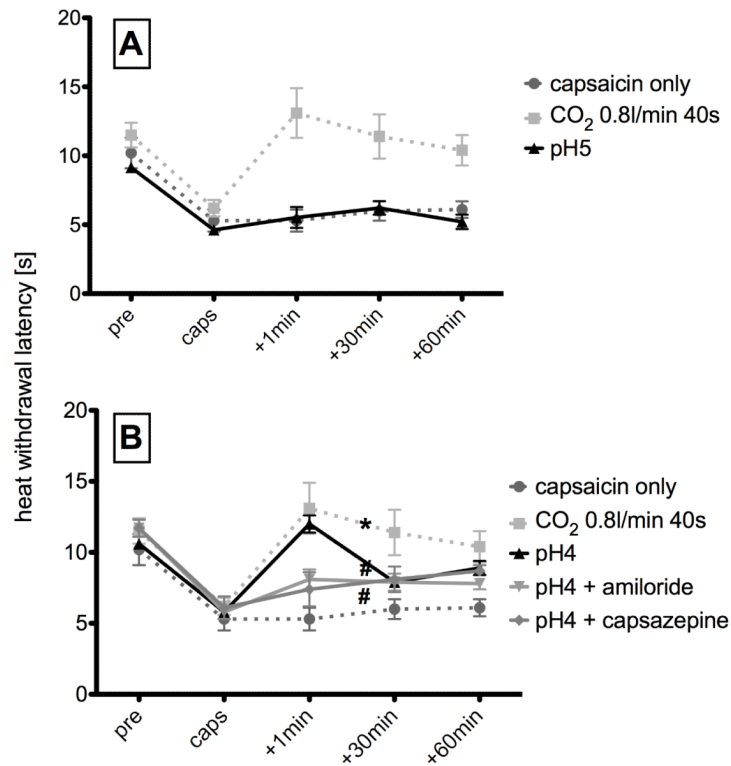


Fig. 5. Effects of pH buffer: A) Intranasal administration of pH5 buffer did not change the decrease in withdrawal latency to a nociceptive heat stimuli after capsaicin sensitization. B) Intranasal administration of pH4 buffer produced a significant ($p < 0.05$, ANOVA) antihyperalgesic effect immediately after application (+1min). This effect was significantly ($p < 0.05$, ANOVA) blocked by pretreatment with the acid-sensing ion channel blocker amiloride (black triangles), as well as by the TRPV₁ blocker capsazepine (rhombs). * indicates statistical significance as compared to “capsaicin only” (two way-ANOVA, $p < 0.05$). # indicates statistical significance as compared to “pH4” (two way-ANOVA, $p < 0.05$).