Investigation of Olfactory CO_2 Detection in Mice.

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Introduction

Physiological concentrations of CO_2 (less than the 4-5% CO_2 in expired air) have been shown to stimulate a small subset of olfactory receptor neurons allowing mice and rats to "smell" low concentration of CO_2 (3,4). While "typical" odorants are known to stimulate olfactory receptors via a cAMP mediated pathway (Fig 1) recent studies indicate that cGMP and the enzyme carbonic anhydrase (CA) are important for the detection of CO_2 (Fig 2)(2,4,5). The objective of this study was to investigate the transduction pathway for CO_2 detection by recording olfactory receptor responses to CO_2 and odorants before and after topical application of L-cis-diltiazem, which inhibits cGMP activated Ca^{2+} channels or niflumic acid, which inhibits Ca^{2+} activated Cl^- channels.

Electro-olfactograms (EOG), which measure summated olfactory receptor responses, were recorded from the surface of the olfactory epithelium in areas known to contain high concentrations of CA (1,4) . EOGs were recorded in response to CO_2 (0-50%) and odorants (amyl acetate, citralva, cyclohexanone, propyl acetate) before and after topical application of the inhibitors.

Figure 1: Odorant Detection

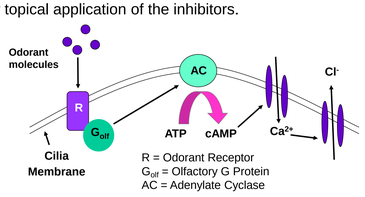
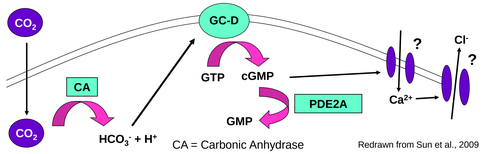


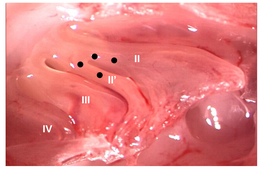
Figure 2: CO_2 Detection



Methods

- Adult mice (C57Bl/6J) were euthanized with pentobarbital and the medial surface of the olfactory epithelium was exposed. EOGs were recorded from endoturbinates II and II' (Fig 3, circles indicate approximate recording sites).
- EOGs were recorded from the surface of the olfactory epithelium using glass electrodes (tip dia. = 10-15 µm) filled with mammalian Ringers.
- CO_2 (0-50%) and odorants were delivered through a multi-barrel olfactometer with a background humidified airflow of 500 ml/min.
- EOG responses to odorants and CO_2 were recorded before and after topical application of Mammalian Ringers, 0.1mM L-cis-diltiazem, or 0.1mM niflumic acid (with <1% DMSO). .

Figure 3: Photomicrograph of mouse olfactory epithelium



Results

Figure 4: Mammalian Ringers (Control)

Typical EOG responses to CO_2 and amyl acetate before (**A**) and after (**B**) topical application of Mammalian Ringers. **C**) Average (±SEM) EOG responses to CO_2 before and after Ringers. **D**). Average (±SEM) EOG response to propyl acetate (PA), amyl acetate (AA), citralva, and cyclohexanone (cyclo) after Mammalian Ringers. * P < 0.05. (N=7)

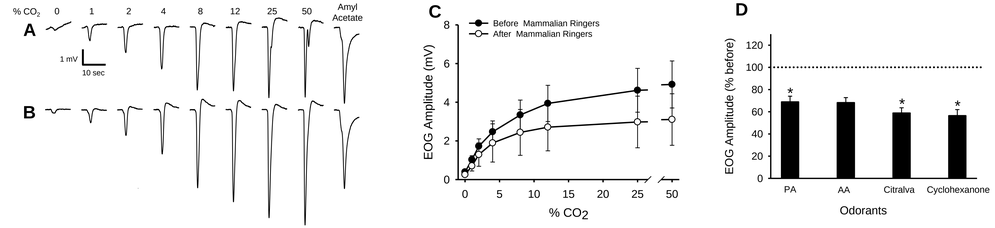


Figure 5: L-cis-diltiazem (Inhibition of cGMP gated Ca^{2+} Channel)

A) Proposed pathway for olfactory CO_2 detection showing the site of action of L-cis-diltiazem. **B)** Average (±SEM) EOG responses to CO_2 before and after L-cis-diltiazem. **C)** Average (±SEM) EOG response to propyl acetate (PA), amyl acetate (AA), citralva, and cyclohexanone (cyclo) after L-cis-diltiazem. * P < 0.05. (N=6)

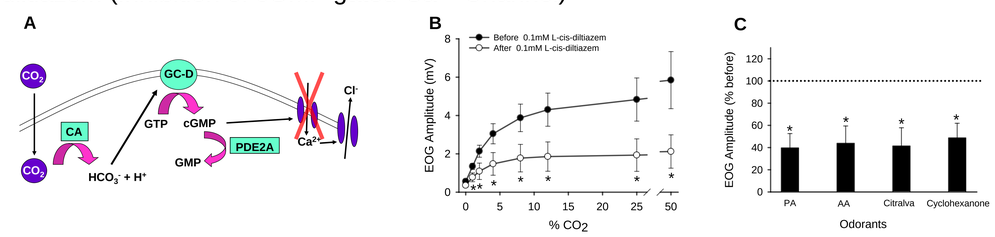


Figure 6: Niflumic Acid (Inhibition of Ca^{2+} gated Cl^- Channel)

A) Proposed pathway for olfactory CO_2 detection showing the site of action of Niflumic Acid. **B)** Average (±SEM) EOG responses to CO_2 before and after Niflumic Acid. **C)** Average (±SEM) EOG responses to propyl acetate (PA), amyl acetate (AA), citralva, and cyclohexanone (cyclo) after Niflumic Acid. * P < 0.05. ** P< 0.001. (N=7)

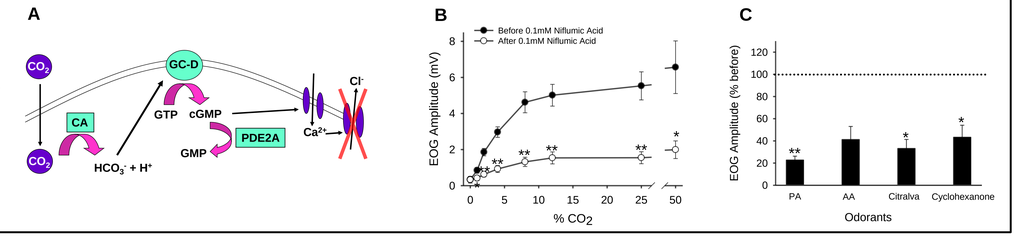
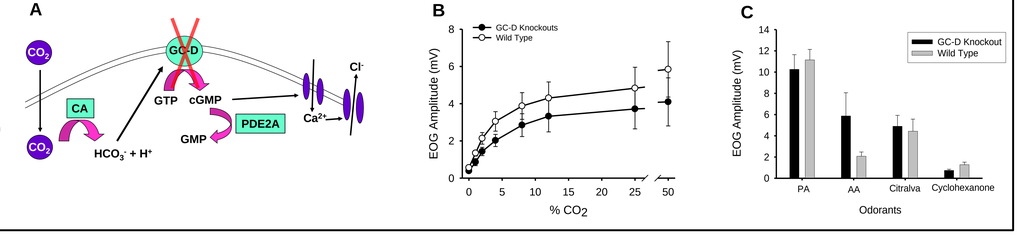


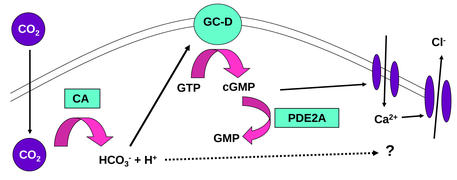
Figure 7: GC-D Knockouts vs. Wild Type Mice

A) Proposed pathway for olfactory CO_2 detection showing the site of GC-D knockout. **B)** Average (±SEM) EOG responses to CO_2 in knockout (filled circle) and wild type (open circle). **C)** Average (±SEM) EOG responses to propyl acetate (PA), amyl acetate (AA), citralva, and cyclohexanone (cyclo) in knockout (black) and wild type (gray). (KO, N=4) (WT, N=6)



Conclusions

- Application of Mammalian Ringers did not significantly affect the olfactory receptor responses to CO_2 but did attenuate the responses to some odorants indicating possible non-specific effects of fluid application.
- Inhibition of cGMP gated Ca^{2+} channels attenuated both the olfactory receptor responses to CO_2 and odorants suggesting that L-cis-diltiazem may inhibit both cAMP and cGMP gated Ca^{2+} channels.
- Inhibition of Ca^{2+} activated Cl^- channels with niflumic acid attenuated both the olfactory receptor responses to CO_2 and odorants showing that Ca^{2+} activated Cl^- channels may play a role in the olfactory CO_2 detection.
- GC-D knockout mice responded to CO_2 at all concentrations tested indicating that the GC-D pathway may not be the only mechanism by which CO_2 stimulates CO_2 -sensitive olfactory neurons (see figure below).



References

1. Coates, E.L. (2001) Olfactory CO_2 chemoreceptors. *Respir. Physiol.*, 129(1-2):219-229.
2. Coates, E.L., Novosat, T.L., Hanson, R.J., Hennessy, S.P., and Kenemuth, J.K. (2009) The Effects of Membrane Permeant and Impermeant Carbonic Anhydrase Inhibitors on the EOG and NMP Responses to CO_2 in Mice. *Association of Chemoreception Sciences*.
3. Ferris, K.E., Clark, R.D., and Coates, E.L. (2007) Topical Inhibition of Nasal Carbonic Anhydrase Affects the CO_2 Detection Threshold in Trained Rats. *Chemical Senses.*, 32:263-271.
4. Hu, J., Zhong, C., Ding, C., Chi, Q., Walz, A., Mombaerts, P., Matsunami, H., and Luo, M. (2007) Detection of near-atmospheric concentrations of CO_2 by an olfactory subsystem in the mouse. *Science.*, 317(5840):953-7.
5. Sun, L., Wang, H., Hu, J., Han, J., Matsunamic, H., and Luo, M. (2009) Guanylyl cyclase-D in the olfactory CO_2 neurons is activated by bicarbonate. *PNAS*. 106:2041-2046.

Acknowledgements

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