Chem. Senses 30 (suppl 1): i196–i197, 2005 doi:10.1093/chemse/bjh182

Mechanisms of Somatosensory Neuronal Sensitivity to Alkaline pH

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Key words: ammonia, BCECF, FURA, nociceptor, polymodal

Introduction

Life for most species is best in a very narrow range of pH. Departures of tissue pH from this narrow range are guarded against by various neural and physiological defense mechanisms. In the past few years, we have gained a much better understanding of what the sensory bases of the defenses against acid insults are. Specifically, several ion channels (TRPV1, Caterina *et al.*, 1997; ASIC3, Waldmann *et al.*, 1997; Ugawa, this issue) have been identified and cloned that respond to even moderately acidic pH. Less is known about the mechanisms monitoring tissue alkalinity. Although tissue acidity is the more common condition that is encountered in pathological situations, there are several conditions that give rise to alkaline tissue. Respiratory alkalosis due to hyperventilation, for instance gives rise to tingling in the extremities and lowered peripheral nerve thresholds (Tenny and Lamb, 1965; Mogyoros *et al.*, 1997). Moreover, alkaline environmental insults to unprotected epithelium such as cornea and nasal mucosa give rise to behavioral defenses such as pain (Acosta *et al.*, 2001) or apnea (Lindberg *et al.*, 1987a) and the physiological defenses of vasodilatation (Izumi and Karita, 1993), tearing and increased mucociliary activity (Lindberg *et al.*, 1987b). In the cranial region these defenses are mediated by the branches of the trigeminal nerve. Ammonia, for instance, activates fibers in the ethmoid branch of the trigeminal nerve (Sekizawa and Tsubone, 1994).

To elucidate the mechanisms underlying trigeminal sensitivity to alkaline pH, responses of cultured rat trigeminal neurons to alkaline stimulation were measured using fluorescence imaging of intracellular calcium, $[Ca^{2+}]_i$ (Kirifides *et al.*, 2004) and intracellular pH (James-Kraacke, 1992). Using this approach, the influence of epithelial permeability barriers and potential stimulatory contributions of the epithelium were eliminated.

Responses and thresholds

Neurons responded to a range of pH stimuli (pH 7.8–10.0 from a baseline of pH 7.35) with reversible increases in $[Ca^{2+}]_i$. While some neurons had thresholds as low as pH 7.8 and as high as pH 10.0, the major portion (50%) of neurons had thresholds of pH 9.0.

What types of neurons are sensitive to alkaline pH?

Consistent with the pain associated with the exposure of the eyes to ammonia and with recordings of ammonia-induced activity in guinea pig nasal polymodal nociceptors (Sekizawa and Tsubone, 1994), we would expect rat polymodal nociceptors to respond to alkalinity. Indeed, pH 9.0 stimulated calcium increases in 15.6% of rat trigeminal neurons that responded to 300 nM CAP. In addition to stimulating polymodal nociceptors, alkaline stimulation (pH 9.0) induced calcium increases in 24.6% cool- and cold-sensitive neurons. Interestingly, although alkaline sensitivity was more strongly associated with the low-threshold, cool neurons, it was not restricted to this population of thermoreceptors. A few high-threshold, cold neurons also responded to pH 9.0. A small group of neurons, 3.5% of the neurons tested with cooling, capsaicin and pH 9.0, only responded to alkaline pH. Thirty-seven percent of neurons did not respond to any level of alkaline stimulation. Thus, while the distribution of sensitivity to alkaline pH appears to be rather promiscuous and not associated with a particular modality, it does not encompass all trigeminal neurons. This suggests that sensitivity to alkaline pH is due to discrete mechanisms and that it is not due to a non-specific mechanism such as membrane perturbation.

pH sensitive mechanisms

In order to elucidate the mechanisms underlying trigeminal neuronal sensitivity to high pH, ion depletion experiments were performed. Responses to pH 9.0 were completely dependent on the presence of extracellular Ca^{2+}. It is therefore likely that an influx of calcium is responsible for alkaline-induced elevations of $[Ca^{2+}]_i$. Diltiazem, an antagonist of L-type voltage-gated calcium channels, suppressed responses to alkaline pH, implying a role for these channels in alkaline sensitivity. The involvement of these channels is further supported by the reported enhancement of calcium currents by alkaline pH (Mironov and Lux, 1991). Indeed, several types of Ca^{2+} channels are positively modulated by high pH (Kiss and Korn, 1999). Sodium channels do not appear to be directly involved with alkaline sensitivity. When extracellular Na was removed (substituted with choline or NMDG) sensitivity to pH 9.0 was unaffected. Although removal of extracellular sodium was a without effect, it is possible that Na^+ channels may contribute to responses to high pH. Above physiological pH, steady state inactivation of Na^+ currents is reduced, which is consistent with alkalosis enhancing excitability (Tombaugh and Somjen, 1998)

Location of the alkaline sensitive site

Neurons that respond to pH 9.0 TRIS, a relatively impermeant buffer, also respond to 30 mM NH_4Cl, pH 7.35. This latter stimulus causes internal alkalinization without raising external pH. By using BCECF, a pH-sensitive dye, to monitor intracellular pH, it is possible to measure internal pH during alkaline stimulation. When exposed to pH 9.0 TRIS, neurons display a modest increase in intracellular pH. Calibration of the BCECF with the proton ionophore CCCP, indicated that intracellular pH only increased to pH 7.7–7.8 during stimulation with external pH 9.0 TRIS. Internal pH during exposure to pH 7.35 NH_4Cl, on the other hand, rose to pH 9.0. Because externally applied pH 9.0 TRIS solutions induced changes in calcium of the same magnitude as internal alkalinization with NH_4Cl, without causing as great an increase in internal pH, it is likely that impermeable bases act primarily on an extracellular site. Exposures to irritants containing ammonium may act both internally and externally, due to the presence of freely diffusible NH_3.

Sensation and coding

Alkaline conditions in the eye and nose are painful. Although only a proportion of polymodal nociceptors is activated by alkaline pH, this may be sufficient to account for the noxious sensation. An additional nociceptive input may come from those cold nociceptors that

are activated. Unless masked by this nociceptive input at high levels of stimulation, it might be expected that alkaline stimulation may interact with the sensation of cooling, due to convergent sensitivity in some cool-sensitive neurons. Although the identity of alkaline-sensitive neurons that were unresponsive to cooling or capsaicin is unclear, it might be expected, that alkaline stimulation may also interact with tactile or other thermal sensations.

References

Acosta, M.C., Belmonte, C. and **Gallar, J.** (2001) *Sensory experiences in humans and single unit activity in cats evoked by polymodal stimulation of the cornea*. J. Physiol., 534, 511–525.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D. and **Julius, D.** (1997) *The capsaicin receptor: a heat-activated ion channel in the pain pathway*. Nature, 389, 816–824.

Izumi, H. and **Karita, K.** (1993) *Reflex vasodilatation in the cat lip elicited by stimulation of the nasal mucosa by chemical irritants*. Am. J. Physiol., 265, R733–R738.

James-Kraacke, M.R. (1992) *Quick and accurate method to convert BCECF fluorescence to pH_i: calibration in three different types of cell preparations.* J. Cell. Physiol., 151, 596–603.

Kirifides, M.L., Kurnellas, M.P., Clark, L. and **Bryant, B.P.** (2004) *Calcium responses of chicken trigeminal ganglion neurons to methyl anthranilate and capsaicin*. J. Exp. Biol., 207, 715–722.

Kiss, L. and **Korn, S.J.** (1999) *Modulation of N-type Ca^{2+} channels by intracellular pH in chick sensory neurons*. J. Neurophysiol., 81, 1839–1847.

Lindberg, S., Dolata, J. and **Mercke, U.** (1987a) *Nasal exposure to airway irritants triggers a mucociliary defense reflex in the rabbit maxillary sinus*. Acta Otolaryngol., 104, 552–560.

Lindberg, S., Dolata, J. and **Mercke, U.** (1987b) *Stimulation of C-fibers by ammonia vapors triggers mucociliary defense reflex*. Am. Rev. Respir. Dis., 135, 1093–1098.

Mironov, S.L. and **Lux, H.D.** (1991) *Cytoplasmic alkalinization increases high-threshold calcium current in chick dorsal root ganglion neurons*. Pflugers Arch., 419, 138–143.

Mogyoros, I., Kiernan, M.C., Burke, D. and **Bostock, H.** (1997) *Excitability changes in human sensory and motor axons during hyperventilation and ischaemia*. Brain, 120, 317–325.

Sekizawa, S. and **Tsubone, H.** (1994) *Nasal receptors responding to noxious chemical irritants*. Respir. Physiol., 96, 34–48.

Tenny, S.M. and **Lamb, T.** (1965) *Physiological consequences of hypoventilation and hyperventilation*. In Fenn, W.O. and. Rahn, H. (eds), Handbook of Physiology. American Physiological Society, Washington, DC, Section 3, pp. 979–1090.

Tombaugh, G.C. and **Somjen, G.G.** (1998) *pH modulation of voltage-gated ion channels*. In Kaila, K. and Ransome, B.R. (eds), pH and Brain function. Wiley-Liss, New York, pp. 395–416.

Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C. and **Lazdunski, M.** (1997) *A proton-gated cation channel involved in acid-sensing*. Nature, 386, 173–177.