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ABSTRACT

This paper outlines the primary factors that affect the neural mediation of behavioral responses to chemical irritants. First, effective irritants must permeate the cornified epithelium or penetrate the mucosa to gain access to nociceptive nerve endings that are present in the skin or mucosa. Physicochemical properties of the irritant/repellent will determine the degree to which an effective concentration can be attained at the nerve endings. Second, endings of specific classes of somatosensory neurons are present in the periphery that, when appropriately stimulated by chemical as well as thermal or mechanical means, signal potential or actual tissue damage by causing pain or sensory irritation. Finally, secondary processes (sensitization, desensitization, and plasma leakage [extravasation]) that modulate the peripheral neural response to potential irritants are discussed.

KEY WORDS

trigeminal, somatosensory, irritation, capsaicin, sensitization

INTRODUCTION

One of the functional subclasses of neurons within the general somatosensory system is the group of neurons that signal pathological internal conditions (inflammation or anoxia) as well as thermal, mechanical, and chemical insults from the external world. An effective course for obtaining nonlethal repellency can target this class of neurons, the nociceptors, using chemical means to cause temporary irritation or pain.

Nociceptors are present throughout the body and integument. This paper will focus on the trigeminal nerve because it is the branch of the somatosensory system that innervates the facial skin as well as the corneas, and oral and nasal mucosae. These regions of an organism are most likely to first encounter a repellent either during respiration, ingestion, or simply on approaching a source of repellent. Moreover, compared to the rest of the integument, the nerve endings serving the mouth, eyes, and nose, are much more accessible to chemical stimulation due to relative differences in skin thickness and permeability.

Three areas of interest that are relevant to the effective stimulation of the trigeminal nerve, vis-a-vis repellency will be discussed. First, the permeability of the target tissue or sensory organs

and the physicochemical properties of a potential repellent are important because it is the interaction of these two factors that determines whether repellents gain access to the nerve endings. Second, the intrinsic sensitivities of different subclasses of nerve endings and how stimuli are encoded by the nervous system determine if a potential repellent causes pain and aversive irritation or whether it will be innocuous. Finally, there are endogenous compounds, which are released during irritation or painful stimulation, that are, in themselves, irritating or cause sensitization to other stimuli.

STIMULUS ACCESS

The receptive endings that serve nociception are present in the integument in the dermal and epidermal layers largely as free nerve endings without secondary specialized structures. In the cornea and nasal mucosa, these same nerve endings are located within several to tens of micrometers beneath the surface, embedded in the epithelium (Silver 1990, MacIver and Tanelian 1993). Throughout the oral mucosa, nerve endings are found in the gingiva, buccal epithelium, and tongue (Dixon 1962). Depending upon which site is considered, (cornea, skin, oral or nasal mucosa), a keratinized epithelium of variable thickness is present. The outermost layer of the keratinized epithelium (stratum corneum) is composed of cells containing large amounts of fibrillar protein as well as intercellular spaces containing high concentrations of lipids. The relative impermeability of this layer of cells is due to its composition.

One of the major determinants of irritancy is access to the nerve endings. If an appropriately impermeable integument, e.g., thickly keratinized epidermis, blocks access of a physiologically active irritant, no sensation occurs. Most skin areas on target animals are, for adaptive reasons, not easily penetrated by many chemical compounds (penetration enhancers notwithstanding). Instead, it is most profitable to target those mucosae in which the nerve endings are separated from the environment by only a few layers of epithelial cells, e.g., nasal and oral mucosae as well as the cornea and the respiratory epithelium. In these areas, there are two routes of access from the exterior of an organism into the vicinity of the nerve endings. Access via the transcellular route involves solubilization in and diffusion through the plasma membrane lipid bilayers and cytosol of the of the epidermis and dermis. Thus, one of the limiting factors of this route of access is lipid solubility or lipophilicity. In addition to this route, an extracellular route is present in the form of the tight junctions that join the first several layers of epithelial cells on their lateral margins. Access to the extracellular space below the epithelial tight junctions is via diffusion through the aqueous phase in the pores of the tight junctions or through the initial layers of extracellular lipids. However, since the tight junctions represent such a small fraction of the area of the mucosal surface through which irritants may diffuse, it is hydrophobic or some amphipathic compounds that will most effectively gain access to sensory endings.

This relationship between lipophilicity and efficacy can be seen in several studies in which the hydrophobic nature of trigeminal stimuli was varied. For both alcohols (Simon and Sostman 1991, Silver et al. 1986) and weak acids (Bryant and Moore 1995), the efficacy of trigeminal nerve stimulation increased as a function of the oil:water partition coefficient or hydrophobicity. Indeed, blocking the tight junctions with lanthanum chloride had no effect on the stimulatory efficacy of the weak acid, pentanoic acid. Thus, the design of irritants must take the hydrophobic

nature of the routes of access into account in order to maximize delivery of the irritant to sensory nerve endings.

NEURAL CODING OF PAIN AND IRRITATION

Most of our understanding of chemically induced sensory irritation comes from experimental studies on the skin in humans and other mammals (Green and Flammer 1988, LaMotte 1983). Recently, we have added to our understanding with studies on the oral and nasal mucosa as well as the cornea and trachea (Fox et al. 1995, Gallar et al. 1993). Stimulation of the nociceptive neurons in each of these areas gives rise to a characteristic sensation or behavioral response. Corneal and dental nociceptors give rise to pain while the stimulation of other tissues and organs can give rise to weaker sensations that elicit blinking, coughing, itching, scratching, as well as outright pain. The physiological basis of each of these sensations is still under investigation. Generally, however, much of the encoding of irritating sensations can be accounted for by certain classes of nociceptors in the periphery.

Nociceptors are classified according to morphological criteria, i.e., conduction velocity and the degree of myelination as well as the modes of stimulation that are effective, i.e., thermonociceptors, mechano-heat nociceptors, etc. The types of nociceptors that are sensitive to chemical stimulation and are known to be involved in the sensations of pain and some forms of irritation are slow conducting, unmyelinated neurons (C-polymodal nociceptors or C-PMN's), that are also sensitive to extremes of heat, cold and mechanical stimulation (Bessou and Perl 1969). The other well-characterized class of chemically sensitive nociceptors is made up of faster conducting, thinly myelinated A-delta fibers. Chemically sensitive A-delta neurons are also sensitive to mechanical and/or thermal stimulation (Perl 1968). In addition to these polymodal nociceptors, it is hypothesized that there are chemonociceptors that are sensitive only to noxious chemical stimulation (LaMotte et al. 1988). These nociceptors are thought to be involved in sensations produced by endogenous compounds.

In the cases cited above, the production of a given sensation can usually be adequately explained by the excitation of a single class of sensory neurons. Sensations such as itch and tingle represent another case where the sensation is produced by excitation of several populations of differentially sensitive neurons. Histamine, which produces itch, and serotonin, which produces pain when injected subdermally, excite overlapping populations of somatosensory nerves, indicating that itch is not simply the excitation of histamine-sensitive nerves. Rather, several classes of nerves must be excited to produce the sensation (Handwerker et al. 1991). Whereas several pain-producing substances may sum through excitation of a single class of nociceptors, it remains to be determined whether other nonpainful, but nevertheless noxious, sensations could be produced by targeted excitation of different classes of nociceptors.

In addition to the classically defined (high threshold) nociceptors, other chemoreceptive neurons have been found that are sensitive to irritating concentrations of acid and carbon dioxide and, therefore, may be involved in producing sensations such as the tingle and burn of carbonation and other weak acids. A class of lingual trigeminal neurons that are sensitive to cooling and not capsaicin has been described that is sensitive to irritating concentrations of weak acids (Bryant and Moore 1995). The fact that these are sensitive to mild cooling and not to capsaicin precludes their

being PMN's. In the lingual branch of the trigeminal nerve, this population of nerves makes up approximately 25% of the neurons sampled (P. A. Moore and B. P. Bryant, Monell Chemical Senses Center, unpubl. obs.). Such a large population of neurons suggests that they are functionally significant. The enhancement of CO_2 irritation by decreased temperatures (Green 1992) supports the involvement of this class of neurons in producing irritating sensations.

The sensitivity of sensory neurons is subject to modulation as a result of direct excitation and by chemical influences from the surrounding tissue. Desensitization, the diminished sensation or responsiveness over time or with repetitive stimulation, is caused by both peripheral and central nervous system (CNS) processes. In some management situations, desensitization may occur and would be undesirable, lessening the effectiveness of a repellent. The opponent process, sensitization, also is due to both peripheral and central processes and could potentially be used to enhance repellent efficacy. Sensitization refers to increased sensitivity to subsequent stimulation by a single mode of stimulus. Another form of sensitization is seen in cross-sensitizing processes in which stimulation by one class of stimuli, i.e., chemical, results in enhanced sensitivity to another class of stimuli. For instance, after extreme thermal (Mizumura et al. 1992) or chemical stimulation by endogenous compounds (bradykinin, serotonin, histamine and prostaglandin E_1) (Davis et al. 1993), some nerve endings become sensitized to thermal and mechanical stimuli. Although the detailed mechanisms of sensitization are not currently understood, various classes of proinflammatory mediators (prostanoids and eicosanoids) as well as histamine and serotonin from mast cells are involved. These compounds are either directly active on the free nerve endings or sensitize the endings to other chemical stimuli (e.g., protons) or to mechanical and/or thermal stimuli. The potential for synergistic or hypersensitizing interactions between chemicals in a repellent or between a chemical repellent and other sensory stimuli, such as thermal or mechanical sensation, has not received adequate attention.

Two other elements play important roles in the sensory response to chemical irritation. First, mast cells, elements of the immune system, are present in the integument, with relatively higher density of these cells in the oral and nasal mucosae (Majeed 1994). Mast cells contain histamine and serotonin as well as enzymes which are involved in the defensive function of the cells. Upon adequate stimulation (chemical or immunogenic), the mast cells degranulate, releasing their contents. Serotonin and histamine induce sensations of itch and pain as well as taking part in the sensitization of nerve endings to other stimulation (Reeh 1994). Recently, a close association of mast cells and nerve endings has been recognized, suggesting a role for the interaction of these two elements in neurogenic irritation and mast cell sensitization of nerve endings (Purcell and Atterwill 1995).

Second, in addition to their function as afferent nerves sending sensory information to the CNS, a subpopulation of somatosensory nerve endings also demonstrates efferent nerve function. This is observed as the release of various neuropeptides such as substance (SP) and calcitonin gene-related peptide (CGRP) in response to adequate and frequently noxious stimulation. Release of these neuropeptides can be stimulated by efferent activity of the CNS as well as by peripheral excitation of the nerve endings. This later case is known as axon reflex and doesn't require any participation of the CNS. Stimulation and depolarization of nerve endings by noxious stimuli spread to other branches of the stimulated nerve causing local secretion of neuropeptides. These neuropeptides are vasoactive, causing changes in the permeability and dilation state of the microvasculature, which results in plasma leakage into the affected tissue. Plasma leakage

delivers elements of the immune system to the affected area as well as diluting the interstitial fluids and removing noxious chemical conditions. In addition, SP plays a role in the sensitization of nerve endings to subsequent stimulation by the inflammatory mediators (Reeh 1994).

Non-nociceptive sensory receptors may also play a role in repellency. Some sensory endings that are sensitive to innocuous thermal stimuli are sensitive to chemical stimuli. For instance, in addition to stimulating polymodal nociceptors, capsaicin stimulates non-nociceptive warm sensitive receptors. Menthol, on the other hand stimulates cooling sensitive receptors, giving rise to cooling sensations. While the bulk of the frankly irritating sensations due to both menthol and capsaicin are likely due to stimulation of polymodal nociceptors, the stimulation of relatively innocuous yet inappropriate thermal sensations in the right behavioral context may be aversive.

CELLULAR MECHANISMS OF NEURONAL ACTIVATION

The sensation of pain or irritation in the CNS is caused by depolarization of the somatosensory nerve endings. Thus, any mechanism that causes dendritic depolarization of a certain subpopulation of nerve endings, the nociceptive endings, can cause pain or irritation. This population of neurons is partially defined by the presence of specific transduction mechanisms that are sensitive to noxious stimuli (extremes of temperature, pressure, pH, or endogenous compounds that induce pain or sensory irritation). Nociceptive neurons are defined as well as by the functional identity of the higher order neurons to which the sensory neurons project. Thus, in addition to activation of nociceptors via stimulus-specific transduction mechanisms, other nonspecific actions of irritants on nerve endings have the potential for causing depolarization of the endings and a sensation of pain or irritation. Four of the known or putative transduction mechanisms of trigeminal neuronal activation are outlined below. With variations in the chemical identity of effective stimuli, these are identical in principal to the mechanisms that give rise to chemical sensitivity in the olfactory and gustatory systems:

1. Specific receptor proteins/ion channels are known or are strongly inferred to mediate the reception of irritants such as capsaicin and menthol (Rang et al. 1991, Swandulla et al. 1987). The activation of some of these receptors is directly coupled to ion channels, the opening of which causes depolarization of the nerve ending. The best understood of these chemonociceptive mechanisms is the receptor/ion channel which mediates the neuronal effects of capsaicin. Capsaicin, as well as many analogues of this vanilloid compound, activate a nonspecific cation channel, causing entry of Na^+ and Ca^{2+} and depolarization of neurons bearing the vanilloid receptor. Prolonged exposure to high concentrations of intracellular Ca^{2+} can lead to desensitization and ultimately to cell death of capsaicin-sensitive neurons (Szolcsanyi 1993). Because it is doubtful that mammals evolved a receptor for capsaicin, it has been hypothesized that there is an endogenous ligand for this receptor (James et al. 1993). Protons are perhaps the best candidate as being an "endogenous vanilloid." Low pH activates the same conductances as capsaicin (Bevan and Docherty 1993) and exhibits synergistic interactions with capsaicin (Martenson et al. 1994). This receptor is a prime target for the induction of repellency.

2. Depolarization of sensory endings is also mediated indirectly by the coupling of specific receptors to ion channels by one of several candidate second messenger systems (cyclic nucleotides and/or phosphoinositides). In these cascades, various enzymes are present (Rang et al. 1991) that are susceptible to activation or inhibition, providing an alternative route for activation of neuronal depolarization. Perhaps the best understood mechanism of this type is the transduction of the endogenous algogen, bradykinin. This nonapeptide is released on cellular injury and activates a specific tachykinin receptor (Rang et al. 1991). Coupled to this is the activation of phospholipases C and A, which affect intracellular Ca^{2+} and thus the state of depolarization.

3. A direct role for ion channels in irritation is strongly suggested by the fact that many ionic stimuli are strong irritants. Green (Gilmore and Green 1993) has demonstrated, in humans, that high concentrations of NaCl are rated as irritating, as well as being salty. KCl, which causes depolarization, elicited pain when applied directly to the exposed skin of a blister (Dash and Deshpande 1976). Specific identification of ion channels, using ion channel blockers to reduce irritation, is lacking. Moreover, the irritation produced by ionic stimuli requires either high concentrations or removal of the epithelial permeability barrier.

4. Nonspecific mechanisms that cause perturbation of the lipid phase of the membranes of nerve endings may lead to dendritic depolarization. This has been proposed to be the mechanism by which some organic solvents induce irritation/pain sensations. It has recently been demonstrated that high concentrations of capsaicin induced conductances in artificial planar lipid bilayers. The experiments indicate that capsaicin molecules alone can induce discrete conductances in artificial lipid bilayers without the intervention of receptors or ion channels (Feigin et al. 1995). This suggests strongly that a similar nonspecific mechanism could occur directly in the lipid phase of nerve endings. Thus, capsaicin or other irritants that induce conductances in dendritic membrane could cause depolarization.

One can see from this list of cellular mechanisms that for a given management problem, a large number of potential targets or entry points into the sensory system of animals exists that can be manipulated biochemically to produce aversive pain and irritation. Indeed, the chemical defenses of many plants and animals take advantage of the intermediate points in these pain pathways to activate nociceptors.

SUMMARY AND RESEARCH NEEDS

1. With a better understanding of the molecular and neural basis of irritancy, molecules can be designed that will be optimized in a variety of relevant parameters such as stimulatory efficacy, access efficacy, and quality and intensity of irritation (pain versus

other less intense irritation). Moreover, the potential for synergistic interactions among components of a repellent mixture should be examined more closely.

2. Taxonomic differences in sensitivity to irritants have been identified. The mechanistic bases of these differences could be better understood to enhance target species specificity.

3. The full range of irritating sensations should be examined. For a given management situation, outright pain may not be necessary. Numbness, tingling, itching, and inappropriate thermal sensations to which the animals do not adapt may be sufficient deterrents.

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