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Sensitization of Nociceptive Ion Channels by Inhaled Anesthetics – A Pain in the Gas?

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Abstract

A remarkable new paper in Molecular Pharmacology (Cornett et al., 2008) shows that the capsaicin-sensitive ion channel TRPV1 is sensitized to activation by chemical and physical stimuli in the presence of inhaled general anesthetics (IGAs). This finding provides another example of an ion channel in which the anesthetic acts to modify channel gating. This may have important clinical implications in view of the role of TRPV1 in nociception.

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The casual reader of a major medical textbook in the 1970s (Cohen, 1975) would have been informed that IGAs were “non-specific agents” that acted “in the bulk lipid phase of the membrane” to “increase membrane fluidity” and thereby act as “membrane stabilizers” to produce unconsciousness. Of course, this was all hogwash. All of the preceding statements in this paragraph are scientifically incorrect, logically inconsistent or linguistic nonsense. Nevertheless, the lipid theory of general anesthesia (Seeman, 1972) had a life of its own, and those early heretics who challenged this medieval orthodoxy were sentenced to hard time in the funding dungeons, before the modern era of molecular pharmacology dawned and brought with it a new enlightenment in this area. The textbooks have since been completely rewritten (Evers and Crowder, 2001).

A recent review of the area (Franks, 2008) shows that our understanding of the molecular pharmacology and neurophysiology of IGAs has come along in leaps and bounds since the 1970s. Detailed analysis has shown that IGAs modulate synaptic transmission in the central nervous system, rather than axonal conduction, and the effects of IGAs are remarkably synapse-specific, and may vary among agents. IGA effects on the brain circuitry involved in sleep (hypothalamic-brain stem-thalamic loops) and memory (hippocampus-amygdala-cortical loops) are currently the subject of intense study (Franks, 2008).

We now know that IGAs are in fact relatively selective, that they act by binding to proteins, and that they do so by occupying small cavities (Bertaccini et al., 2007). In many cases, the relevant proteins are ion channels, and the effect of IGA binding to these allosteric sites is to alter the gating of these channels by physiological stimuli, presumably by altering the thermodynamic equilibrium between open and closed states of the channel.

There are numerous important examples of ion channels in which gating has been shown to be modified by IGAs at clinically relevant concentrations. These include the γ-aminobutyric acid (GABA)-A receptors, the NMDA subtype of glutamate receptors, voltage-gated Na+ channels, the “twin-pore” K+ channels (all reviewed in Franks and Lieb, 2004 and Franks, 2008), and now the TRPV1 channel.

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The existence of IGA binding sites as cavities within ion channels was first suggested by mutagenesis experiments (Mihic et al., 1997), but numerous IGA binding sites have now been studied in proteins of known structure using high-resolution techniques (Bhattacharya et al., 2000). The binding of IGAs in these cavities is stabilized by a combination of hydrophobic interactions and hydrogen bonding interactions. In addition to these modest enthalpic contributions there is also a significant entropic component of the free energy of IGA binding that is provided by the displacement of bound water molecules from within the cavity (Bertaccini et al., 2007).

TRPV1 was first identified and has been most thoroughly studied in nociceptive neurons in the sensory system (Caterina et al., 1997), in which TRPV1 basically functions as a “molecular sensor” and as an integrator of a variety of physical and chemical stimuli including heat, capsaicin (the active ingredient of hot peppers) and low pH. In fact, protons and capsaicin act at interacting allosteric sites, and this is the origin of the remarkable synergy between capsaicin and fruit juices or vinegar in certain spicy foods (think of this molecular interaction the next time you enjoy (?) a lamb vindaloo). In addition to capsaicin, TRPV1 is activated by a variety of endogenous and exogenous substances, such as the endocannabinoids and eicosanoid lipids. These stimuli can act, either independently or in concert, to promote channel activation. TRPV1 also appears to be sensitized and modulated via G-protein- and phospholipase C-coupled signalling cascades, that can be initiated by, for example, bradykinin (Ramsey et al., 2006). In the peripheral nervous system, TRPV1 is one of the molecular entities responsible for “thermal hyperalgesia”, the phenomenon by which thermal stimuli are more painful under conditions of tissue injury and inflammation (Caterina et al., 2000; Davis et al., 2000).

In their elegant paper, Cornett et al. (2008) demonstrate that IGAs are another class of substances that can sensitize TRPV1 to activation, not only by capsaicin but also by low pH and heat. The effect of isoflurane (ISO) on TRPV1 channels is to shift the dose-response curve (in ligand-gated ion channels, this is essentially akin to the activation curve in voltage-gated ion channels) for capsaicin and other activators to the left. This is entirely analogous to the well-known effect of ISO on GABA-A and glycine receptors, in which the drug effectively enhances the actions of low concentrations of neurotransmitter, presumably by altering the relative thermodynamic stability of the open and closed states so that the open state becomes more energetically favoured, resulting in an increase in the channel open probability. The synergistic interaction between ISO and other stimuli of TRPV1 is not

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unprecedented: it has already been shown that one TRPV1-agonist at sub-effective concentrations can lower the response threshold for the channels to other agonists (Tominaga et al., 1998). It is therefore conceivable that higher concentrations of IGAs might be agonists of the channel *per se*, as is the case for isoflurane with GABA-A receptors, for example.

We know from previous studies that the endogenous pro-inflammatory and noxious substance bradykinin sensitizes and activates nociceptors and that TRPV1 is one of the targets of the bradykinin-initiated signalling cascade, contributing to the modulation of nociceptor sensitivity after tissue injury (Chuang et al., 2001). In fact, TRPV1-deficient mice do not develop thermal hyperalgesia in response to inflammation. Another important effect demonstrated in the paper by Cornett and colleagues that fits nicely into the current picture of TRPV1 is that PKC activation (or activation of bradykinin receptors) can enhance the action of IGAs on TRPV1.

A detailed molecular mechanism has not been presented, but from a clinical perspective this effect is intriguing, especially in view of another recent finding from the Ahern laboratory, in which they show that another TRP channel, TRPA1 (a relative of TRPV1 expressed in sensory neurons) is activated by IGAs (Matta et al., 2008). The data of Cornett et al. would suggest that clinically used IGAs can exhibit pro-nociceptive effects, at least under conditions of tissue injury and inflammation. During surgery, the release of bradykinin from damaged tissue would be expected to sensitize TRPV1 and thereby enable additional activation of sensory neurons by IGAs. The modulation and activation of TRPV1 should therefore probably be regarded as an unwanted side-effect of IGAs on the nociceptive system.

Although corresponding data in a clinical setting is currently lacking, it seems conceivable that IGAs, by directly activating or modulating TRPV1 and its relative TRPA1 in nociceptive neurons, can cause a clinically significant sensitization of nociceptors. The resulting enhancement of neurogenic inflammatory processes might be expected to persist well beyond the intra-operative period, and thus prove relevant in the etiology of the post-operative and persistent pain that can be a significant hedonic and economic factor in delaying patient recovery. If this concept were supported by clinical data, the new information might revolutionize the understanding among anesthesiologists of the consequences of the intra-operative use of IGAs and other agents that are routinely used to induce and maintain surgical anesthesia. Indeed, if future studies are able to substantiate long-lasting side

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effects of GAs on the nociceptive system that might contribute to persistent pain, then TRPV1 and/or TRPA1 antagonists might prove to be powerful analgesic adjuncts for the prevention and treatment of post-operative pain.

Some interesting questions remain: is the effect of IGAs on TRPV1 a direct action of the anesthetic on the channel? This seems likely, as the effect is very rapid and is seen in excised membrane patches - there is certainly ample precedent for an effect on channel gating via direct binding. What is the nature of the binding site for IGAs and how does it relate to the complex gating mechanisms of TRPV1? Clearly these are all questions worthy of future investigation.

In recent years, there has been a decline in work on anesthetics at the molecular level, in favor of research that is “translational” in nature. The observations of Cornett et al. (2008) demonstrate clearly that there is still much to be learned about the effects of IGAs on the nervous system, and also show that the distinctions drawn between “basic” and “translational” research can be arbitrary and short-sighted. The lesson to be gleaned here is that the pursuit of new knowledge can often reap an unexpected harvest. By pursuing an understanding of TRPV1 at the molecular level, these researchers may have uncovered new insights into the age-old problem of post-surgical pain that have profound implications for patient care.

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