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Local Anesthetics

A New Hydrophilic Pathway for the Drug-receptor Reaction

WHERE, how, and by what pathway local anesthetics produce their effects, at least those related to inhibition of action potentials in axons, have curiously only recently become established dogma in Anesthesia. Now we are presented with data in the current issue of this journal that support a challenge to some of our long-standing assumptions about the pathways by which local anesthetics may approach their binding site.[1,2]

Shortly after Hodgkin and Huxley used voltage-clamping to posit the existence of specific Na channels in the giant axon of the squid, *Loligo*, Weidmann observed cocaine- and procaine-induced reductions in the amplitude and peak rate of rise of action potentials in isolated Purkinje fibers.[3] Weidmann speculated that cocaine and procaine might promote inactivation of Na channels in cardiac muscle. Taylor reported that procaine had no effect on the resting membrane potential or on the Na equilibrium potential of squid axons.[4] Rather, procaine specifically inhibited Na currents. Taylor also observed that procaine had a less robust action at inhibiting K currents and commented that this latter action would tend to counteract procaine "block" of nerves.

These observations only slowly made there way into the anesthesia literature. A contemporary textbook[1] stated that local anesthetics might work through interference with electrical depolarization of nerves or "humoral actions" (competing with acetylcholine for receptor binding).[5] Strichartz described use-dependent block in 1973, and he reviewed local anesthetic pharmacology in ANESTHESIOLOGY in 1976, documenting the data that discredited those theories of local anesthetic action that did not involve binding of the drugs to Na channels.[6] A year later, Hille provided an explanation (and a drawing) of the pathways by which charged and neutral local anesthetics might reach the drug receptor on the Na channel within the plasma membrane.[7] Yet, a contemporary textbook continued to provide two theories of local anesthetic action: one involving Na channels and the other involving conformational changes in lipoproteins and membrane expansion.[8] There was also a comment that local anesthetics could produce their effects ". . . internally or externally at the channel opening," despite the lack of evidence to support an external site. In 1990, another review of local anesthetic pharmacology appeared in ANESTHESIOLOGY that again emphasized the central importance of drug binding to Na channels.[9] Meanwhile, textbooks published in that decade continued to speculate about uncharged local anesthetics causing membrane expansion and, of all things, "decreasing the diameter of the sodium channel."[10,11]

Presently, the local anesthetics in clinical use are (with the exception of the neutral compound benzocaine) tertiary amines that under physiologic conditions exist in a mixture of protonated and neutral forms. The charged forms appear to be more potent than the neutral forms once they gain access to the local anesthetic binding site on the cytoplasmic "side" of the conducting pore of the Na channel.[6,9] Quaternary, obligatorily charged local anesthetics have been used for 30 yr to define the site of drug action and to determine the "active form" of local anesthetics.[6,9] The assumption has long been made that if QX-314 (a compound similar to lidocaine in every way except that it has a third ethyl moiety on its terminal amine nitrogen that renders it positively charged under all conditions) or any other quaternary compound is placed extracellularly, the positive charge will prevent it from permeating the plasma membrane. When applied to the cytoplasmic side of neural membrane QX-314 will potently produce both tonic and frequency-dependent block. Nevertheless, accepted dogma states that QX-314 should not have an effect if used for local or regional anesthesia in patients because there would be no way for it to approach the local anesthetic receptor.

We are now presented with strong evidence that our dogma regarding QX-314 is in conflict with the facts. First, there were two reports that extracellular quaternary local anesthetics could inhibit Na channels.[12,13] Vanilloid receptor agonists were observed to potentiate local anesthesia.[14,15] Next, Lim *et al.* reported that application of QX-314 could produce long-lasting nerve blocks in animals.[16] Binshtok, Bean, and Woolf reported that extracellular application of QX-314 with capsaicin, a compound that promotes opening of transient receptor potential vanilloid subtype I (TRPV1) channels, produced greater inhibition of membrane Na currents than either compound administered by itself.[17] Local anesthetics were added to the list of drugs and conditions that activate TRPV1 channels.[18]

This Editorial View accompanies the following articles: Binshtok AM, Gerner P, Oh SB, Puopolo M, Suzuki S, Roberson DP, Herbert T, Wang C-F, Kim D, Chung G, Mitani AA, Wang GK, Bean BP, Woolf CJ: Co-application of lidocaine and the permanently charged sodium channel blocker QX-314 produces a long-lasting blockade in rodents. ANESTHESIOLOGY 2009; 111:127–37; and Ries CR, Pillai R, Chung CCW, Wang JTC, MacLeod BA, Schwarz SKW: QX-314 produces long-lasting local anesthesia modulated by transient receptor potential vanilloid receptors in mice. ANESTHESIOLOGY 2009; 111:122–6.

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In this edition of ANESTHESIOLOGY Binshtok *et al.* extend these observations with a report that coapplication of lidocaine with QX-314 promotes a long-persisting regional anesthesia, presumably by lidocaine's promoting activation of TRPV1 channels through which QX-314 gains entry to the cytoplasm.[17] Meanwhile Ries *et al.* used activators and inhibitors of TRPV1 to provide strong evidence that this channel represents the most likely pathway by which QX-314 might breach the plasma membrane.[18] But, are these reports consistent with what is known about the biophysical characteristics of TRPV1 channels?

It has long been known that capsaicin, the pungent principal of hot chili peppers, can induce burning pain when injected into the skin and, paradoxically, can serve as an analgesic.[19] Furthermore, embryonic exposure to capsaicin leads to failure of development of small diameter nociceptor sensory afferent neurons and an inability to express pain-related behaviors. For these reasons, the 1997 cloning of the capsaicin receptor, now termed TRPV1, represented a major breakthrough in understanding the transduction of noxious stimuli in the periphery.[20] Since that time, a superfamily of TRP channels (now including six vanilloid-type receptor-channels [TRPV1-6]) has been recognized to transduce a variety of physical and chemical stimuli, including heat, cold, osmotic strength, mechanical force, and Ca^{2+} ion depletion. Several of the TRPV channels along with TRPM8 and TRPA1 have been implicated in thermal sensation; however, evidence from two lines of TRPV1-null mice suggest that TRPV1 is required for certain pathologic hyperalgesic states, but not for physiologic thermal sensation. This apparent segregation of pathologic and physiologic pain responses has renewed interest in the development of both selective TRPV1 antagonists and agonists as potential novel analgesic agents.[19]

Much is now known about TRPV1 structure-function relationships. Identified domains of TRPV1 bind capsaicin, sense heat, respond to activation by protons, and respond to inflammatory signals. Biochemical and functional data reveal the receptor to be a tetramer of subunits, each possessing six transmembrane (TM) helices and a putative reentrant pore lining loop between TM5 and TM6 markedly similar to the structure of the voltage-gated K channel (Kv) family. Activated TRPV1 is nonselective for the passage of monovalent cations, but it is five to nine times more permeable to Ca^{2+} ions over monovalent ions. Ca^{2+} entry through TRPV1 is essential for desensitization of the channel and may play a role in triggering nociceptive neuron death with prolonged exposure.

The recent excitement surrounding the appearance of crystal structures of K channels is due in part to their support of concepts derived from decades of biophysical measurements of ion permeation using electrophysiological and ion flux measurements on native and cloned K channels.[21] Single-file passage, ion dehydration, and coordination of ions with but a very few residues in a "selectivity filter" all remain components of ion selectivity in the revised models of K channels. The similarities between TRPV1 and Kv channel structures implied by experimental and theoretical comparisons naturally led to the view that ion selectivity in TRPV1 is also a relatively static feature reflecting rigid structural domains of the channel. This view, however, is challenged by recent observations of anomalous permeation of TRPV1 by much larger molecules. Moreover, evidence also suggests that Kv channels can exhibit dynamic ion selectivity under certain conditions, suggesting the general view of a static immutable selectivity filter needs rethinking.

The first report that anomalously large molecules could pass through TRPV1 came from a chance observation that the styryl dye FM1-43 could gain entry into a variety of sensory cells and neurons through TRP channels.[22] Only cell lines heterologously expressing TRPV1 would accumulate the dye in response to activation. Since that report, other investigators have documented that molecules ranging from large dyes (YO-PRO1, MQAE), antibiotics (gentamycin), and large organic cations (NMDG, tetraethylammonium) will permeate TRPV1 channels.[23–25] Inspired by these observations, Chung *et al.* made comprehensive biophysical assessments of TRPV1 permeability and selectivity under conditions of prolonged activation by capsaicin, heat, and other agonists.[26] These authors documented a progressive decrease in channel selectivity with increasing activation time and/or agonist concentration yielding passage of large dyes and organic cations through TRPV1. Thus, extended activation of TRPV1 as might occur during conjoint application of capsaicin (or lidocaine or thermal stimulation) and QX-314 could readily result in permeation of QX-314 to the cytoplasmic compartment, where it potently blocks Na channels.

Based on atomic radii, the limiting size of the selectivity filter of TRPV1 under acute activation has been estimated at 6–10 Å, whereas the crystal structure of lidocaine suggests it to be a longitudinal structure (greater than 30 Å) with lateral dimensions of 4.7×6.7 Å.[27] Thus, even without dynamic "dilation" of the selectivity filter, it is conceivable that a lidocaine derivative could enter a neuron *via* an activated TRPV1 permeation pathway. Any agonist-induced increase in the effective diameter of the permeation pathway would further facilitate entry of such molecules.

Is the concept that the structurally homologous Kv channel has a relatively static "selectivity filter" regulating ion permeation reasonable? Armstrong *et al.* have reported a dramatic decrease in selectivity of Kv in squid axons when K^+ ions were excluded from the solutions bathing both internal and external surfaces of the channel, suggesting lability of the selectivity filter in Kv channels.[28] Furthermore, it is relevant to note that a requirement to obtain viable crystals of Kv channels is the

presence of permeating ions (*e.g.*, K^+ or Rb^+) to presumably stabilize the channel in a physiologic condition.[21] Thus, it appears that the selectivity filter of the closely homologous Kv channel family may be as labile as that of TRPV1.

If we accept the notion that quaternary local anesthetics can gain entrance to neurons *via* activated TRPV1 channels, will this mechanism have any clinical importance? In other words, is it likely that clinicians will direct QX-314 molecules into neuronal cytoplasm and onward to Na channels to produce persisting regional analgesia? The answers to these questions will come only after much more is known about the effectiveness and safety of both vanilloid receptor activation and of the quaternary anesthetics.

A final question might be: if this proposed pathway for QX-314 to cross the plasma membrane is accepted by basic scientists, will it take 5, 10, or 20 yr before it is accurately described in our textbooks?

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