Vanilloid (Capsaicin) Receptors in Health and Disease

Arpad Szallasi, MD, PhD

Key Words: Vanilloid receptor VR1; Capsaicin; Resiniferatoxin; Anandamide

Abstract

The cloned vanilloid (capsaicin) receptor subtype 1 (VR1) integrates multiple noxious stimuli on peripheral terminals of primary sensory neurons. The initial excitation of these neurons is followed by a lasting refractory state, traditionally termed desensitization*, that has clear therapeutic potential. Capsaicin is used to relieve neuropathic pain, uremic pruritus, and bladder overactivity. The ultrapotent vanilloid resiniferatoxin, now in phase 2 clinical trials, has improved tolerability. A less recognized human exposure to high capsaicin concentrations may occur by pepper sprays used in law enforcement.*

Evidence is mounting that VR1 expression is not restricted to sensory neurons. From the olfactory bulb to the cerebellum, VR1-expressing neurons are present in a number of brain nuclei, where they might be activated by anandamide. VR1 presence also was demonstrated in nonneuronal tissues. These discoveries place VR1 in a much broader perspective than pain perception and enhance the potential for unforeseen side effects, especially following prolonged vanilloid therapy. The expression of VR1 is plastic and down-regulated during vanilloid therapy, which might have a pivotal role in desensitization. Good evidence suggests altered VR1 expression in various disease states. This recognition not only may provide novel insights into pathogenesis but also may prove useful in diagnosis.

Most pathologists are familiar with capsaicin **Figure 1** only as the compound responsible for the piquancy of hot pepper. Related compounds include piperine, the principal irritant in black pepper, and zingerone, present in ginger.[1] Connoisseurs of hot, spicy food also know the predominant pharmacologic actions of capsaicin from personal experience: the initial burning sensation that it causes quickly disappears, a phenomenon known by pharmacologists as *desensitization*,[2] and then the tongue becomes insensitive to various noxious stimuli, including heat. Some may also experience profuse perspiration during a spicy meal, scientifically referred to as *gustatory sweating*.[3] A selective responsiveness to capsaicin has long been recognized as a functional signature of polymodal sensory neurons.[4] Specific binding of resiniferatoxin (RTX) (Figure 1), an ultrapotent capsaicin analog isolated from the dried latex of the cactus-like plant *Euphorbia resinifera*, provided the first biochemical proof for the existence of a specific capsaicin receptor.[5,6] Since RTX and capsaicin share a vanillyl moiety as a structural motif essential for bioactivity but differ dramatically in the rest of the molecule, these 2 classes of pungent compounds were referred to collectively as *vanilloids*, and their membrane recognition site was termed the *vanilloid receptor*.[6] Once a pharmacologic oddity, capsaicin has become a cover story in both *Nature*[7] and *Science*[8] with the molecular cloning of a complementary DNA encoding a functional vanilloid (capsaicin) receptor, termed VR1 (vanilloid receptor subtype 1), and the subsequent development of VR1-deficient mice. Antibodies and molecular probes to detect human VR1 are now available. There is rekindled interest in VR1 as a therapeutic target, and new findings enhance the potential for using altered VR1

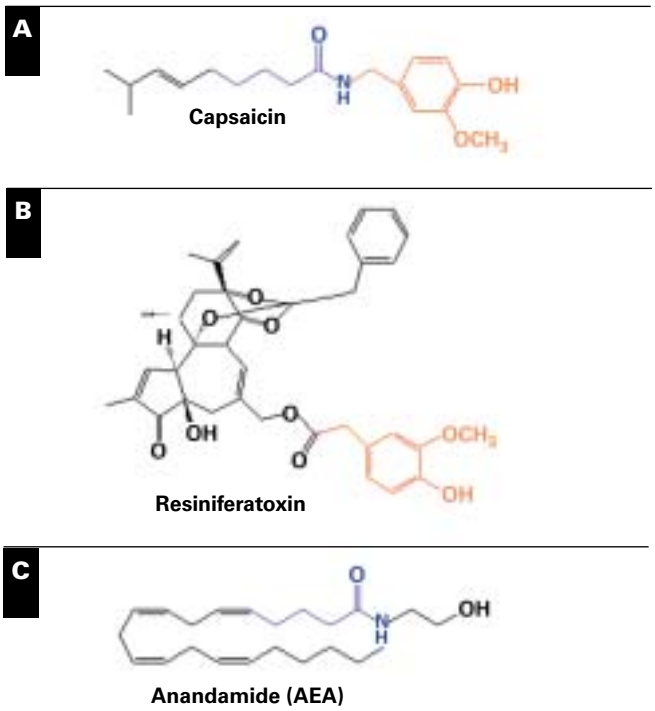

Figure 1 Typical vanilloid structures with the similarities highlighted in red (capsaicin and resiniferatoxin) or blue (capsaicin and anandamide). **A**, Capsaicin, the pungent principle in hot pepper. **B**, The ultrapotent capsaicin analogue resiniferatoxin, isolated from the latex of the cactus-like plant *Euphorbia resinifera*. **C**, The endogenous cannabinoid anandamide, which also acts as a full agonist at the human vanilloid receptor, hVR1. AEA, *N*-arachidonoylethanolamine.

expression in the molecular diagnosis of various disease states. The molecular pharmacology of VR1[6,9,10] and the hopes and realities of vanilloid therapy[6,11-13] have been reviewed extensively. The focus of this review is on the implications of recent advances in vanilloid research for diagnostic pathology.

Molecular Biology of the VR1

VR1 is a serpentine membrane protein **Figure 2** with 6 complete transmembrane spanning segments (S1 to S6) and an extracellular loop linking S5 to S6, known as the P-loop, which is believed to include the channel pore.[14] Although this P-loop shows limited similarity to Shaker-type potassium channels, VR1 functions as a nonselective cation channel with high permeability to Ca^{2+}.[6,7,9] VR1 is a distinct relative of the TRP (transient release potential) superfamily of store-operated calcium channels.[15] Related proteins are expressed throughout the evolutionary tree from the worm *Caenorhabditis elegans* (in fact, OSM-9 was the first member of this family to be cloned[16]) to *Drosophila* to birds, where these channels have a role in sensory transduction. The capsaicin recognition domain, however, seems to be an evolutionary recent addition to VR1, inasmuch as only mammals respond to capsaicin.[2,4] This recognition has been put to practical use in the development of capsaicin-containing bird seeds to repel squirrels.

Although the vanilloid receptor originally was defined as the molecular target for vanilloid compounds,[1,4] the emerging concept is that VR1 principally functions as a noxious heat (>43°C) sensor on sensory neurons.[7,17] Capsaicin and mild acidification lower the temperature threshold for channel activation.[7] Half-maximal potentiation by protons occurs at pH 7.0.[18] Further acidification (pH 6 or less) induces a slowly activating current through VR1 even at room temperature.[18] Potentiation by protons of heat-evoked VR1 activation and direct activation by protons of VR1 are mediated by distinct molecular recognition sites.[18] The pH dependence of thermal activation is related to the side-chain charge of the residue at position 600 (normally a glutamine), whereas direct activation occurs via position 648. Both of these residues are localized to the P-loop, as is the structural determinant (position 646) of ruthenium red (RR) sensitivity.[14] Pathologists know RR as an inorganic histochemical dye. Years ago, RR was, however, extensively used by pharmacologists on an empiric basis to block various channels including the capsaicin receptor.[19] In site-directed mutagenesis experiments, replacement of D646 by asparagine rendered VR1 insensitive to RR blockade.[14] Capsaicin activates VR1 differently from either heat or protons.[20] The vanilloid-binding domain on VR1 is yet to be identified. It is most likely intracellular[21] and is believed to be present either on the N-terminus or the sixth transmembrane segment.[22] Since monomeric VR1 expressed in *Xenopus* oocytes recapitulates the positive cooperative nature of VR1 activation by vanilloids,[7] it is likely that more than 1 vanilloid-binding site exists on a single receptor.

The predicted molecular mass of VR1 is 95 kd.[7] This is at variance with the much larger (390-kd) molecular target size of native vanilloid receptors in radiation inactivation experiments.[23] To resolve this apparent contradiction, VR1 was postulated to exist in a multimeric form, possibly as a tetramer.[6] This is entirely consistent with the heterogeneity of vanilloid-evoked currents.[24] In support of this model, an electrophoretic gel analysis of VR1 showed oligomers with a tetramer being the most prominent form.[25] Functional analysis of VR1 using a dominant negative mutant also is consistent with tetrameric stoichiometry for the native vanilloid receptor.[22] The heterogeneity of vanilloid-evoked currents also may reflect the existence of VR1 isoforms. Indeed, a model for the genomic organization of the *hVR1* gene predicts skipping of exon 7 to yield divergent VR1 splice variants.[26]

The recently cloned human VR1 (hVR1)[27] shows 92% homology to rat VR1 (rVR1) and is located at chromosome 17p13, downstream of the nephropathic cystinosis gene.[28] Despite this high degree of homology, rat and human VR1 show differences in activation,[29] warranting caution when extrapolating experimental data from rats to humans.

The molecular regulation of *VR1* gene expression is unknown. In some tissues, the *VR1* gene seems to be regulated developmentally. For example, in rat cardiomyocytes, VR1 messenger RNA (mRNA) is first detected on day 14 of embryonal life, but then it disappears after postnatal day 30.[30] In the newborn rat, but not in the adult, the expression of the *VR1* gene seems to be dependent on the presence of nerve growth factor.[2,6]

Extended Family of Proteins Related to VR1

Initially sparked by a hunt for the capsaicin receptor, an expanding family of related proteins has been identified within the past 3 years **Figure 3** and **Image 1**. The second family member to be isolated shares 49% amino acid identity with VR1. Although originally cloned from rat brain, this protein is expressed widely throughout the body. This receptor is activated by high temperatures (>52°C) but not by vanilloid ligands or protons; therefore, it was termed *vanilloid receptor–like protein 1*, or briefly rVRL1.[31] Somewhat confusingly, the independently cloned mouse orthologue of VRL1 was defined as growth factor–regulated channel,[32] abbreviated GRC. Recently, a corresponding human orthologue (hVRL-1) was isolated from a myeloid cell line, and the *hVRL-1* gene was mapped to chromosome 4q25.[33] There is preliminary evidence that, though also activated by high temperatures, VRL1 functions primarily as a mechanosensitive receptor.

An N-terminus truncated form of VR1, referred to as the *vanilloid receptor 5′ splice variant* (VR.5'sv), was detected both in neuronal tissues and leukocytes.[34] It is believed to arise from alternative splicing of VR1 mRNA transcripts.[26] The activator of VR.5'sv is yet to be identified. A mechanosensitive stretch-inhibitable cation channel (SIC) was derived from kidney, where it is thought to be activated by hypertonic conditions, ie, cell shrinkage, and inhibited by cell swelling.[35] Furthermore, SIC is colocalized with VR1 on primary sensory neurons, where it is believed to participate in the mediation of pain produced by hypertonic stimuli.[36] The SIC channel is highly homologous to VR1 but lacks part of the N-terminus of VR1 with the ankyrin repeat elements. Also, it has a different intracellular carboxyl terminus. VR1 and SIC seem to be derived from 2 related but independent genes, but the *SIC* gene is yet to be localized.[26] The alternative hypothesis

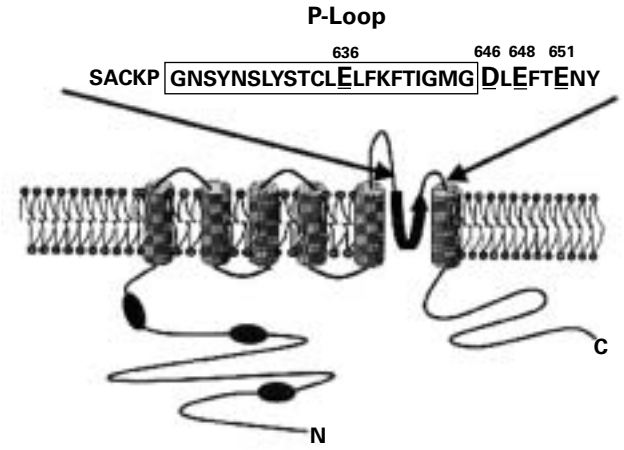

Figure 2 Putative membrane topology of the vanilloid receptor subtype 1, VR1. In the N-terminus, the closed oval symbols represent the 3 ankyrin repeat domains. On top is depicted the deduced amino acid sequence for the proposed pore-forming region (boxed area), also known as the P-loop, that connects the fifth and sixth transmembrane spanning segments. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; S, serine; T, threonine; Y, tyrosine. Acidic residues are underlined. From Garcia-Martinez et al.[14] Reprinted with permission.

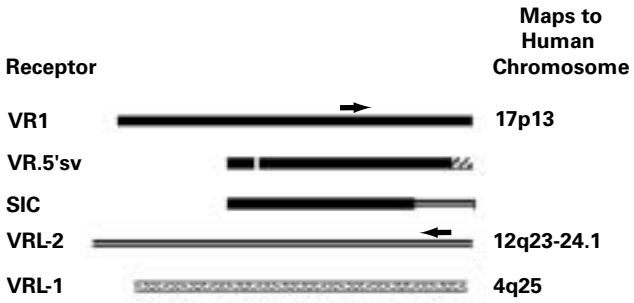

Figure 3 Schematic representation and chromosomal localization of vanilloid receptor subtype 1 (VR1) and related family members. The chromosomal localization in humans is noted on the right. SIC, stretch-inhibitable cation channel; VR.5′sv, vanilloid receptor 5′ splice variant; VRL-1, vanilloid receptor–like protein 1; VRL-2, vanilloid receptor–like protein 2. Courtesy of Simon N. Tate, PhD.

postulates that the SIC protein is the result of *trans*-splicing events between VR1 and VRL-1 mRNAs.[26]

The fifth member of the family, called *vanilloid receptor–like protein 2* (VRL-2), was isolated from the kidney using in silico analysis of expressed sequence tag databases.[33] VRL-2 shares 46% identity with VR1 and is strongly expressed in the airways, kidney (distal tubules), and salivary glands.[33] It also is present on mononuclear cells,

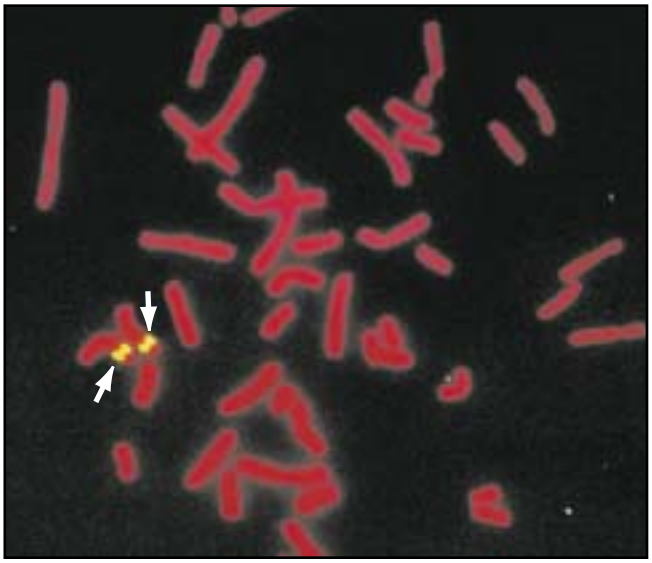

Image 1 Fluorescence in situ hybridization experiment demonstrates the position of vanilloid receptor–like protein 2 on chromosome 12q23-24.1 (arrows). Courtesy of Simon N. Tate, PhD.

as well as on sympathetic and parasympathetic fibers innervating the blood vessels, sweat glands, and arrector pili smooth muscles of the skin.[33] VRL-2 is believed to be osmotically regulated,[37] hence its alternative name, *vanilloid receptor–related osmotically activated channel*. VRL-2 was cloned simultaneously in various laboratories, regretfully resulting in a bewildering array of names such as OTRPC4 or Trp12.[38] The human *VRL-2* gene was localized to chromosome 12q23,[33] a region already linked to bipolar affective disorder.[39] The VR1 family of proteins has been extended to include the calcium transport protein, derived from rat intestine,[40] and the epithelial calcium channel, cloned from rabbit intestine and placenta.[41] Human orthologues for both genes have been identified.

Generally speaking, members of the VR1 family of proteins are highly conserved in their transmembrane spanning regions but are very heterogeneous with regard to the N-terminal amino sequences (Figure 3, Image 1). Therefore, the current model is that these proteins are cation channels that show similar ion selectivity but differ in regulatory mechanisms of channel gating. It is important to note that, as yet, VR1 remains the only receptor to be activated by vanilloid compounds. Although presently overshadowed by VR1, the other members of the family clearly deserve further study with regard to their functional roles. As described herein, VR1 exists in multimeric forms, and one interesting possibility to explore is that VR1 may form heteromultimers with its relatives.

As described above, VR1 is most similar to the TRP superfamily of store-operated calcium channels.[15] Recently, the TRP Nomenclature Committee has proposed that a new TRP channel subfamily be created, called TRPV, where V stands for "vanilloid." As of today, this subfamily has 5 members, namely VR1 (now TRPV1), VRL-1 (TRPV2), VRL-2 (TRPV4), CaT1 (TRPV5), and CaT2 (TRPV6). The name TRPV3 is yet to be assigned. It remains to be seen, however, if this new nomenclature will gain broad acceptance.[42]

Tissue Distribution of VR1 and the Physiologic Functions of VR1-Expressing Cells

For decades, there had been a consensus in the literature that the expression of the (then putative) capsaicin receptor was restricted to a specific subset of primary sensory neurons,[4] namely to those giving rise to thin, unmyelinated axons, also known as C fibers. Later capsaicin-sensitivity was extended to include some neurons with thin myelinated (A delta) fibers and vagal neurons with perikarya in nodose ganglia.[2,43] Although capsaicin-evoked responses were described in several brain nuclei, as well as in various nonneuronal tissues, those were regarded as "nonspecific" actions, ie, not mediated by the vanilloid receptor.[6] Even the original report by Caterina and colleagues[7] on the cloning of VR1 described the presence of VR1 mRNA exclusively in dorsal root ganglia. The concept of capsaicin being a "selective sensorotoxin," however, can no longer be maintained in the light of the recent demonstration of VR1 throughout the whole neuroaxis[44,45] and in a surprising variety of nonneural tissues.[46-48]

VR1 on Primary Sensory Neurons

Vanilloid-sensitive primary sensory neurons are bipolar. The peripheral fibers innervate the skin, the mucosal membranes, and almost all internal organs.[2,4,6] Activation of the nerve endings results in the release of proinflammatory neuropeptides and the generation of impulses that are transmitted to the spinal cord via the central fibers, where they are perceived as pain or itch. Of these neuropeptides, substance P (SP) and calcitonin gene–related peptide deserve special attention.[2] SP, acting on neurokinin NK1 receptors expressed by dorsal horn neurons, has an important role in pain transduction.[49] Capsaicin depletes SP, which is thought to contribute to the long-lasting analgesic actions of capsaicin administration.[2,4,6] Interestingly, SP also was shown to act as a growth factor for keratinocytes, and it was speculated that an increased production of SP by dermal vanilloid-sensitive nerves might contribute to the pathogenesis of psoriasis.[50] Indeed, capsaicin creams were reported to be beneficial in patients with psoriasis.[51]

Calcitonin gene–related peptide aids in the maintenance of mucosal integrity in the gastrointestinal tract.[52] In animal models, capsaicin protects against gastric ulcer formation and minimizes tissue damage during experimental colitis.[53] Capsaicin also was suggested to exert antimicrobial actions against *Helicobacter pylori*,[54] which promises to be an added benefit for patients with an ulcer. Given the postulated role of inflammation in the pathogenesis of colon carcinoma,[55] capsaicin should be protective against colon tumorigenesis. Indeed, a 60% reduction by dietary capsaicin was reported in the incidence of colonic adenocarcinoma induced by azoxymethane in the rat.[56] This finding implies a role for vanilloids in the prevention of human colon cancers. One study, however, links hot pepper consumption to enhanced risk for gastric carcinoma,[57] and the jury is still out with regard to the safety of high-dose dietary capsaicin.[6]

In the rat, vanilloid-sensitive nerves have 2 distinct subdivisions,[58] a peptidergic subdivision, characterized by the coexpression of VR1 with neuropeptides, and a purinergic one, possessing the adenosine triphosphate (ATP)-gated ion channel $P2X_3$. It is unclear whether this segregation also applies to humans, but the coexpression of VR1 with $P2X_3$ on afferents innervating the human urinary bladder **Image 2** has already been demonstrated.[59] ATP has long been thought to function as a neurotransmitter in the bladder, participating in reflex responses that control volume and voiding. In support of this theory, $P2X_3$-null mice showed decreased emptying frequency and increased bladder volume.[60] Interestingly, megalobladder is one of the very few abnormalities that develop in animals whose vanilloid-sensitive nerves have been depleted by neonatal capsaicin treatment (unpublished observation). Taken together, these findings imply a pivotal role for purinergic vanilloid-sensitive afferents in the micturition reflex. In passing, it is worth mentioning that some rats develop nonhealing skin ulcers following neonatal capsaicin treatment.[61] As discussed, SP released from vanilloid-sensitive nerves may act as a growth factor on keratinocytes, and it was speculated that the wounds may reflect the ablation of SP-containing nerve endings by capsaicin.[61]

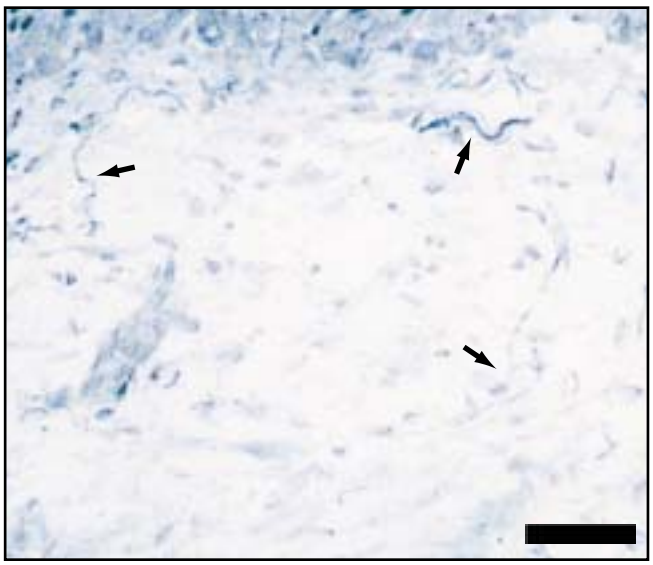

Image 2 Vanilloid receptor subtype 1–immunoreactive fibers (arrows) in the suburothelium of human bladder (cystoscopic biopsy material; scale bar = 40 μm). From Yiangou et al.[59] Reprinted with permission.

VR1-Expressing Neurons in the Brain

In the rat, VR1-positive neurons were found throughout the whole neuraxis, including all cortical areas (in layers 3 and 5), several regions of the limbic system (such as the hippocampus, amygdala, and the habenula), striatum, hypothalamus, centromedian and paraventricular thalamic nuclei, reticular formation, locus ceruleus, cerebellum, and inferior olive.[44] VR1-immunoreactive cells also were found in the third and fifth layers of the human parietal cortex.[44] With the exception of the anterior hypothalamus (preoptic area), which is firmly linked to the hypothermic action of capsaicin, the biologic role of VR1-positive brain neurons is enigmatic.[45] In the substantia nigra, there is a complete overlap between VR1-positive and tyrosine hydroxylase–immunoreactive cells, identifying vanilloid-sensitive nigral cells as monoaminergic.[44] In animal models, RTX has a powerful antiemetic action.[62] Moreover, there is anecdotal evidence linking capsaicin to appetite control.[6,45]

VR1-Expressing Cells in Nonneural Tissues

Arguably the most exciting, but no doubt the most controversial, recent finding in the vanilloid field is the demonstration of VR1-positive cells in nonneural tissues, negating the central dogma of VR1 being a sensory neuron–specific receptor. In animals, the presence of VR1 protein and/or mRNA already has been described in the bladder,[47] kidney,[46] spleen,[46] heart,[29] stomach,[63] and mast cells.[64] As yet, the only human tissue reported to express VR1 protein is the epidermis.[48] The presence of VR1 mRNA was demonstrated by reverse transcriptase–polymerase chain reaction (RT-PCR) in the human bladder.[65] The list of VR1-positive human tissues is expected to show a rapid growth in the foreseeable future.

Now there is good evidence that the VR1 protein in the rat urothelium represents a functional vanilloid receptor.[47] Capsaicin evokes calcium uptake by urothelial cells in culture. As in sensory neurons, this response in the urothelium is potentiated by acidification and is abolished by the competitive VR1 antagonist capsazepine.[47] Urothelial cells also release ATP and nitric oxide (NO) in response to

capsaicin treatment.[47] This is interesting because both ATP and NO are known to stimulate vanilloid-sensitive nerve endings. Furthermore, ATP was shown to enhance the effect of capsaicin on VR1-gated ion currents by an allosteric mechanism.[66] It therefore was speculated that urinary bladder epithelial cells might assume a sensory role that allows them to respond, in concert with neighboring vanilloid-sensitive afferents, to noxious environmental stimuli.[47] As already alluded to, the transitional epithelium of the bladder is exposed to extremely high capsaicin or RTX concentrations during intravesical vanilloid therapy.[6] Although no significant morphologic changes were found in the bladder biopsy materials of patients undergoing topical RTX treatment for detrusor hyperreflexia,[67] the authors were not aware of the vanilloid-sensitive nature of the urothelium itself. Clearly, these patients need further scrutiny with regard to possible toxic effects of vanilloids on transitional epithelial cells.

The function of VR1 on smooth muscle cells of the bladder is puzzling.[47] It also is unclear whether the VR1 mRNA detected in the kidney[46] was derived from the urothelium of the renal pelvis or another cell type.

The presence of VR1 on mucous neck cells of the proliferative zone of gastric glands suggests a role for VR1 in the control of mucosal cell proliferation.[63] Capsaicin was reported to have a marked effect on immune functions in experimental animals (reviewed by Szallasi and Blumberg[68]). Although it was thought that this action was indirect (ie, mediated by neuropeptides released from vanilloid-sensitive nerve endings and acting on lymphocytes), the demonstration of vanilloid receptors in mast cells,[64] combined with the presence of VR1 mRNA in the spleen,[46] raises the intriguing possibility that VR1 is involved directly in immunoregulatory functions.

Exogenous Activators of VR1 and Putative Endogenous Vanilloids

The current thinking about VR1 is that it functions primarily as a noxious heat sensor.[9] Vanilloid ligands are believed to act by reducing the heat-activation threshold of VR1.[6,9] This theory makes perfect sense with regard to VR1 on peripheral terminals of C fibers and provides an elegant explanation to the common observation that capsaicin evokes a hot, burning sensation on the human tongue. Protons have long been the focus of attention as "small stimulants of capsaicin-sensitive sensory nerves."[69] Now it is clear that protons have a dual action on VR1: mild acidification only potentiates heat effects, but a further drop in pH probably results in direct channel gating.[18] It is believed that acids produced under hypoxic conditions activate cardiac sensory nerves via VR1 and thereby contribute to the excruciating pain of angina pectoris.[70] This model also applies to muscle pain and gives a rationale to explain the beneficial effects of capsaicin creams in relieving muscle cramps. Although protons may have a role in inflammatory pain, several eicosanoid products of lipoxygenases and leukotriene B_4 also were shown to activate VR1.[71]

The N-terminus of VR1 contains kinase consensus sequences.[7,9] Indeed, protein kinase C was reported to activate VR1 directly,[72] and it may be speculated that VR1 (at least on certain cells) is not a ligand-gated but rather an enzyme-gated channel. This hypothesis may have far-reaching implications by coupling various algesic agents, such as bradykinin, to signaling via VR1.

Of putative endogenous vanilloid receptor activators, anandamide is the most controversial.[73-75] Anandamide (arachidonoylethanolamine) is best known as an endogenous ligand of cannabinoid receptors (endogenous marijuana, if you will): it is formed on demand by phospholipase D from phospholipid precursors and is inactivated by a combination of facilitated cellular reuptake and enzymatic hydrolysis.[75] Although initially anandamide was found to be a full agonist of VR1 in some bioassays,[76] it seemed to be a low-affinity one, resulting in understandable reluctance to assign any physiologic relevance to this action.[74] Eventually, it was found that the apparent affinity of VR1 for anandamide is strongly influenced by assay conditions. Indeed, under appropriate assay conditions, anandamide activates VR1 at concentrations at which it also operates the cannabinoid receptor CB1.[75] The emerging concept is that the anandamide recognition domain on VR1 is intracellular and, to activate VR1, anandamide needs to be carried through the membrane by a specific anandamide membrane transporter.[75] It may be of physiologic relevance that NO enhances the activity of this transporter and thereby enhances the apparent affinity of VR1 for anandamide.[75] As described, NO is released from bladder epithelial cells upon capsaicin stimulation.[47] Thus, an activation cascade could be visualized in which VR1-expressing urothelial cells and nerves engage in reciprocal VR1 potentiation by releasing NO and maybe also other transmitters. Although the controversy whether anandamide is really an endovanilloid in the periphery or not has no closure in sight,[74] this compound represents an attractive candidate for being an activator of VR1 in the central nervous system, where neither heat nor protons are likely to gate VR1, and anandamide shows remarkable colocalization with VR1-positive brain nuclei.[45] Indeed, anandamide recently has been shown to activate VR1 in rat hippocampus slice preparations.[77]

In passing, it also should be mentioned that a growing number of drugs have been reported to either activate or block VR1 in in vitro assays. Examples include cocaine and

the intravenous anesthetic agent propofol.[78] Whether an interaction with VR1 in vivo does have a role in the pharmacologic actions of these drugs remains to be determined.

Vanilloids in Clinical Practice

There are exhaustive reviews on the current clinical uses and future therapeutic potential of vanilloid drugs.[6,11-13] At present, only VR1 agonists are used in clinical practice, but data obtained in VR1-deficient mice support the clinical potential of antagonists.[8,79] The main current indications for capsaicin therapy are intractable neuropathic pain, spinal detrusor hyperreflexia, and bladder hypersensitivity.[6,11-13] In controlled clinical trials, a beneficial effect of capsaicin also was demonstrated in cases of interstitial cystitis, vasomotor rhinitis, migraine, cluster headache, pruritus associated with chronic renal failure, and lichen simplex chronicus and for patients undergoing hemodialysis.[6,11-13] Moreover, there is anecdotal evidence to suggest a role for capsaicin in the treatment of Guillain-Barré syndrome, psoriasis, burning mouth syndrome, and notalgia paresthetica, just to cite a few examples.[6] Capsaicin given by systemic administration is a powerful drug in animal models of human diseases, but topical capsaicin has never lived up to the expectations in humans for 2 main reasons. First, capsaicin is very painful despite attempts to minimize discomfort by coadministration of local anesthetics, and this side effect makes many patients quit treatment.[6] Second, it is difficult to deliver capsaicin through the skin or mucous membranes to the nerve endings at sufficiently high concentrations to cause desensitization.[6] A new approach to enhance the clinical effectiveness of topical capsaicin is to use very high concentrations under regional anesthesia.[80] The safety of this approach, however, needs to be carefully evaluated in the light of the recent demonstration of the presence of VR1 on keratinocytes.[48] When neuropathic pain is well-localized, perineural infiltration by capsaicin of the involved nerves may be attempted.[81]

In experimental animals, RTX is clearly superior to capsaicin because it induces lasting desensitization with minimal initial pain.[6,68] At present, RTX is undergoing phase 2 multicenter clinical trials on both sides of the Atlantic, with promising results for treatment of detrusor hyperreflexia and bladder hyperreflexia.[82-87] RTX causes much less discomfort than does capsaicin at therapeutically useful concentrations. In the pioneering study of Cruz and colleagues,[88] patients rated the discomfort evoked by intravesical RTX (50-100 nmol/L) instillation as 1 to 3 on a relative scale of 1 to 10, where 10 corresponds to the pungency of capsaicin. The maximal cystoscopic capacity almost doubled (from 182 to 330 mL) following RTX treatment and, even more important, 9 of the 12 incontinent patients in the study became dry or at least showed significant improvement.[88] Subsequent clinical studies[83,87,89] not only confirmed the efficacy of intravesical RTX treatment but also reported increases in maximal bladder capacity up to 500% following a single instillation.[90] It is important to note that the beneficial effect of a single RTX treatment seems to last as long as 12 months.[82,88] This is of special importance as spinal detrusor instability usually is a sequel to multiple sclerosis or traumatic spinal cord injury, and, thus, these patients are restricted in their mobility and need transportation for office visits.

VR1 in the Practice of Pathology

It is clear that physicians working at pain clinics and neurologists treating patients with neurogenic bladder dysfunction should have a basic knowledge of the pharmacology of vanilloid-sensitive nerves. The relevance of VR1 for pathologists, especially for those who work in a private practice setting, is less self-explanatory.

As discussed, capsaicin and RTX already are in clinical use, and it is predicted that, with the development of novel vanilloid drugs, an increasing patient population will be exposed to VR1 ligands. Consequently, pathologists will encounter biopsy or surgical material with increasing frequency from patients exposed to vanilloids and will have to address the question whether the observed histologic changes are an effect of treatment. There is unequivocal experimental evidence that capsaicin causes gross neurotoxic effects when given systemically at high doses.[2,4] Nerve degeneration also was demonstrated in human skin following intradermal capsaicin injection.[91] The question whether topical capsaicin or RTX ablates nerve endings at therapeutically useful concentrations is still subject to heated debate. Recently, a painstaking electron-microscopic analysis of bladder biopsy specimens showed a modest, but statistically significant, increase in mean nerve diameter following intravesical capsaicin treatment,[92] implying a selective loss of the smallest unmyelinated fibers. There was a concomitant increase in the number of axons per nerve profile, consistent with nerve injury by capsaicin followed by regeneration and sprouting. Currently, it remains unclear whether this toxic action is a side effect of capsaicin that can be avoided by the use of improved vanilloid drugs or is an essential component of desensitization.

Light microscopy of bladder biopsy material reveals that the urothelium is unremarkable following intravesical RTX instillation and the lamina propria shows occasional mononuclear inflammatory cells.[67] An electron-microscopic evaluation of the bladder biopsy material showed no significant ultrastructural changes.[67] Dasgupta and colleagues[93] observed 20 patients for 5 years who had received an

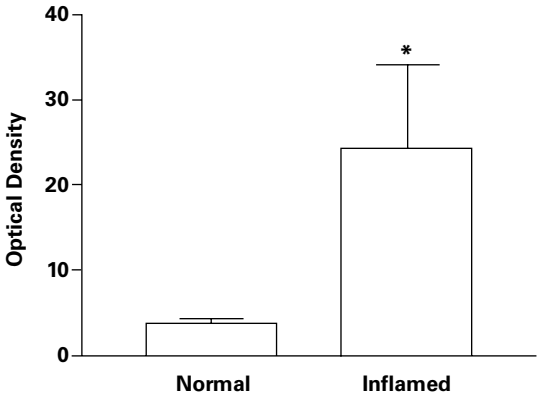

Figure 4 Increased vanilloid receptor subtype 1 immunoreactivity in Crohn disease. A representative experiment. Optical density values are expressed as mean ± SEM. *P = .0023. Courtesy of Praveen Anand, MD.

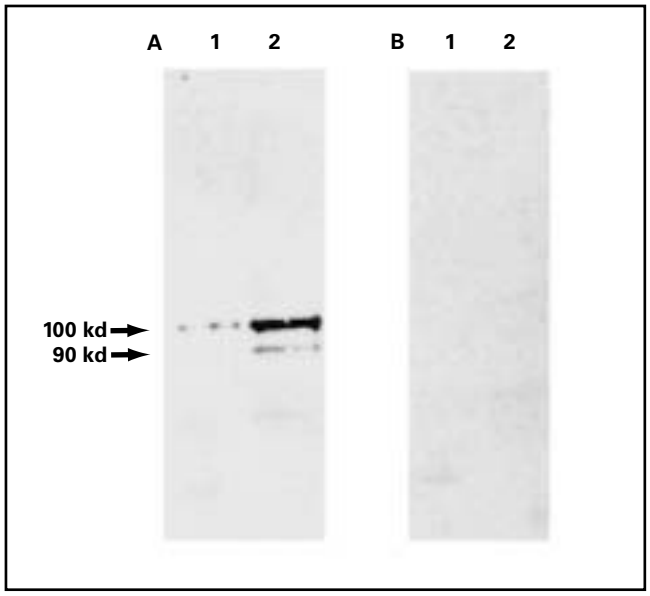

Image 3 Increased vanilloid receptor subtype 1 (VR1) immunoreactivity in Crohn disease. **A**, Western blot analysis, using an antibody to human VR1, shows a marked increase in the optical density of the 100-kd band, corresponding to VR1 protein, in inflamed human intestine (lane 1) compared with control samples (lane 2). The minor 90-kd band probably represents a nonglycosylated form of the VR1 receptor. **B**, Preabsorption with excess VR1 peptide completely abolished the signals generated by these bands. Courtesy of Praveen Anand, MD.

average of 6 intravesical treatments with capsaicin, and none of the bladder biopsies showed any premalignant (metaplasia or dysplasia) or malignant (in situ or invasive carcinoma) changes. Although all available evidence suggests that both capsaicin and RTX are safe when given intravesically,[82-87] the recent demonstration[47,65] of the effects of VR1 on bladder epithelial and smooth muscle cells necessitates further studies in the human bladder.

As described, topical capsaicin is used in clinical practice to ameliorate neuropathic pain and pruritus. Moreover, capsaicin is an active ingredient in a number of over-the-counter creams used by millions on a daily basis for the relief of muscle ache or cramps.[68] It is known that exposure of the skin to high capsaicin concentrations may cause severe, acute irritation referred to as *Hunan hand*. Although the effects of long-term exposure are unknown, the presence of VR1 on keratinocytes[48] is, nevertheless, provocative.

One outstanding clinical question, which may be of relevance for pathologists, concerns the observation that many patients with a suitable neurologic profile do not respond to vanilloid treatment.[87] It is entirely feasible that responders vs nonresponders reflect individual variability in VR1 expression. If so, the evaluation by a pathologist of tissue biopsy material for VR1 mRNA using RT-PCR might provide the clinician with important information about treatment options.

There is good evidence to suggest altered VR1 expression in various disease states. As shown in **Figure 4**, **Image 3**, and **Image 4**, VR1-like immunoreactivity is enhanced in colonic nerve fibers of patients with active inflammatory bowel disease.[94] There are no observable differences between tissues taken from patients with Crohn disease and from those with ulcerative colitis.[94] Hypertrophic nerve bundles in hypoganglionic and aganglionic bowel segments of patients with Hirschsprung disease show intense VR1-like immunopositivity, whereas normoganglionic regions are similar to those in control subjects.[95] Capsaicin induces a motility response in the esophagus of healthy volunteers but not in patients with Barrett esophagus.[96]

Finally, forensic pathologists should be aware that capsaicin may induce a severe and potentially fatal asthma attack in asthmatic patients.[97] Therefore, it is recommended that health care workers wear a mask during application of capsaicin creams and that those with asthma also have an albuterol inhaler available. Capsaicin-containing sprays,[98] fondly called Sergeant Pepper, are used by law-enforcement officials to subdue violent criminals, and in the state of California they also are marketed for self-defense as a "cop-in-a-can." The use of capsaicin spray by a police officer has been implicated in the death of a criminal with asthma.

Summary and Conclusions

Vanilloid receptor subtype 1 (VR1) is a sensor of noxious stimuli on peripheral terminals of primary sensory neurons. Ligands that desensitize or block VR1 have a proven clinical potential to relieve pruritus or neuropathic

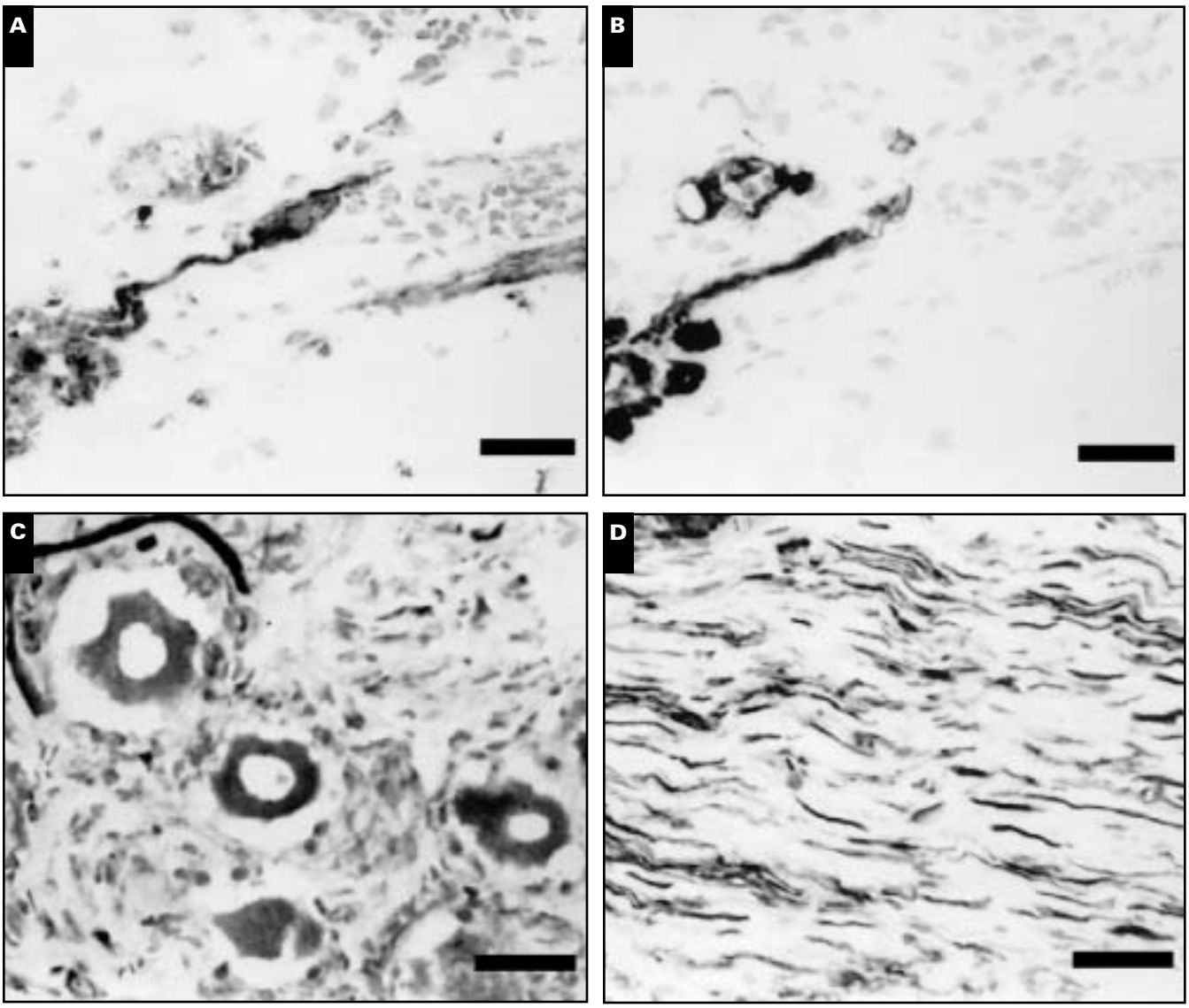

Image 4 Increased vanilloid receptor subtype 1 (VR1) immunoreactivity in Crohn disease. **A**, VR1-immunoreactive nerves are associated with the submucosal plexus in inflamed (Crohn disease) human intestine. **B**, Peripherin immunoreactivity in the same structure as for VR1. VR1-immunoreactive neurons in human dorsal root ganglia (**C**) and their fibers in the dorsal spinal root (**D**). Scale bar = 40 µm. From Yiangou et al.[94] Reprinted with permission.

pain. Such drugs also are beneficial in the treatment of detrusor hyperreflexia and the overactive bladder, where the afferent limb of the micturition reflex is mediated by vanilloid-sensitive C fibers. The recent demonstration of VR1 in various brain nuclei, as well as in nonneuronal cells such as the urothelium or keratinocytes of the epidermis, places VR1 in a much broader clinical context than mere pain perception and enhances the potential for unforeseen side effects during long-term treatment with vanilloid drugs. Capsaicin and RTX are natural products that activate and then desensitize VR1. They are powerful drugs in animal models of human disease. Unfortunately, their clinical usefulness is limited. Capsaicin is pungent. RTX is superior to capsaicin in its tolerability profile, but commercial RTX preparations have an inconsistent clinical efficacy for unclear reasons. Novel, synthetic VR1 ligands are under development, and it is hoped that these drugs will be devoid of the shortcomings of naturally occurring vanilloids. With the emerging evidence that altered VR1 expression has a role in the pathogenesis of various disease states, continued exploration of VR1 expression in health and disease is expected to provide pathologists with novel diagnostic tools and may identify new therapeutic targets for vanilloid drugs.

From the Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO.

Address reprint requests to Dr Szallasi: Dept of Pathology and Immunology, Division of Clinical Pathology, Washington University School of Medicine, Box 8118, 660 S Euclid Ave, St Louis, MO 63110.

Acknowledgments: I thank John Pfeifer, MD, PhD, for reading the manuscript and providing useful comments. I am grateful to Praveen Anand, MD, and Simon N. Tate, PhD, for the illustrations they provided.

References

1. Szolcsányi J. Capsaicin type pungent agents producing pyrexia. In: Milton AS, ed. *Handbook of Experimental Pharmacology*. Berlin, Germany: Springer; 1982:437-478.
2. Holzer P. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol Rev*. 1991;43:143-201.
3. Lee TS. Physiological gustatory sweating in warm climate. *J Physiol (Lond)*. 1954;124:528-542.
4. Buck SH, Burks TF. The neuropharmacology of capsaicin: a review of some recent observations. *Pharmacol Rev*. 1986;38:179-226.
5. Szallasi A, Blumberg PM. Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, by dorsal root ganglion membranes. *Brain Res*. 1990;524:106-111.
6. Szallasi A, Blumberg PM. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol Rev*. 1999;51:159-211.
7. Caterina MJ, Schumacher MA, Tominaga M, et al. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 1997;389:816-824.
8. Caterina MJ, Leffler A, Malmberg AB, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science*. 2000;288:306-313.
9. Caterina MJ, Julius D. Sense and specificity: a molecular identity for nociceptors. *Curr Opin Neurobiol*. 1999;9:525-530.
10. Tominaga M, Julius D. Capsaicin receptor in the pain pathway. *Jpn J Pharmacol*. 2000;83:20-24.
11. Dasgupta P, Fowler CJ. Chilies: from antiquity to urology. *Br J Urol*. 1997;80:845-852.
12. Kim DY, Chancellor MB. Intravesical neuromodulatory drugs: capsaicin and resiniferatoxin to treat the overactive bladder. *J Endourol*. 2000;14:97-103.
13. Szallasi A. Vanilloid receptor ligands: hopes and realities for the future. *Drugs Aging*. 2001;18:561-573.
14. Garcia-Martinez C, Morenilla-Palao C, Planells-Cases R, et al. Identification of an aspartic residue in the P-loop of the vanilloid receptor that modulates pore properties. *J Biol Chem*. 2000;275:32552-32558.
15. Harteneck C, Plant TD, Schultz G. From worm to man: three subfamilies of TRP channels. *Trends Neurosci*. 2000;23:159-166.
16. Colbert HA, Smith TL, Bargmann C. OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. *J Neurosci*. 1997;17:8259-8269.
17. Tominaga M, Caterina M, Malmberg AB, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron*. 1998;21:531-543.
18. Jordt SE, Tominaga M, Julius D. Acid potentiation of the capsaicin receptor determined by a key extracellular site. *Proc Natl Acad Sci U S A*. 2000;97:8134-8139.
19. Amann R, Maggi CA. Ruthenium red as a capsaicin antagonist. *Life Sci*. 1991;49:849-856.
20. Welch JM, Simon SA, Reinhart PH. The activation mechanism of rat vanilloid receptor 1 by capsaicin involves the pore domain and differs from the activation by either acid or heat. *Proc Natl Acad Sci U S A*. 2000;97:13889-13994.
21. Jung J, Hwang SW, Kwak J, et al. Capsaicin binds to the intracellular domain of the capsaicin-activated ion channel. *J Neurosci*. 1999;19:529-538.
22. Kuzhikandathil EV, Wang H, Szabo T, et al. Functional analysis of capsaicin receptor (vanilloid receptor subtype 1) multimerization and agonist responsiveness using a dominant negative mutation. *J Neurosci*. 2001;21:8697-8706.
23. Szallasi A, Blumberg PM. Molecular target size of the vanilloid (capsaicin) receptor in pig dorsal root ganglia. *Life Sci*. 1991;48:1863-1869.
24. Liu L, Simon SA. Capsaicin-induced currents with distinct desensitization and Ca^{2+} dependence in rat trigeminal ganglion cells. *J Neurophysiol*. 1996;75:1503-1514.
25. Kedei N, Szabo T, Lile JD, et al. Analysis of the native quaternary structure of vanilloid receptor 1. *J Biol Chem*. 2001;276:28613-28619.
26. Xue Q, Yu Y, Trilk SL, et al. The genomic organization of the gene encoding the vanilloid receptor: evidence for multiple splice variants. *Genomics*. 2001;76:14-20.
27. Hayes P, Meadows HJ, Gunthorpe MJ, et al. Cloning and functional expression of a human orthologue of rat vanilloid receptor-1. *Pain*. 2000;88:205-215.
28. Touchman JW, Anikster Y, Dietrich NL, et al. The genomic region encompassing the nephropathic cystinosis gene (CTNS): complete sequencing of a 200-kb segment and discovery of a novel gene within the common cystinosis-causing deletion. *Genome Res*. 2000;10:165-173.
29. McIntyre P, McLatchie LM, Chambers A, et al. Pharmacological differences between the human and rat vanilloid receptor 1 (VR1). *Br J Pharmacol*. 2001;132:1084-1094.
30. Dvorakova M, Kummer W. Transient expression of vanilloid receptor subtype 1 in rat cardiomyocytes during development. *Histochem Cell Biol*. 2001;116:223-225.
31. Caterina M, Rosen TA, Tominaga M, et al. A capsaicin receptor homologue with a high threshold for noxious heat. *Nature*. 1999;398:436-441.
32. Kanzaki M, Zhang YQ, Mashima H, et al. Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. *Nat Cell Biol*. 1999;1:165-170.
33. Delany NS, Hurle M, Facer P, et al. Identification and characterization of a novel human vanilloid receptor–like protein, VRL-2. *Physiol Genomics*. 2001;4:165-174.
34. Schumacher MA, Moff I, Sudanagunta SP, et al. Molecular cloning of an N-terminal splice variant of the capsaicin receptor: loss of N-terminal domain suggests functional divergence among capsaicin receptor subtypes. *J Biol Chem*. 2000;275:2756-2762.
35. Suzuki M, Sato J, Kutsuwada K, et al. Cloning of a stretch-inhibitable nonselective cation channel. *J Biol Chem*. 1999;274:6330-6335.
36. Schumacher MA, Jong BE, Frey SL, et al. The stretch-inactivated channel, a vanilloid receptor variant, is expressed in small-diameter sensory neurons in the rat. *Neurosci Lett*. 2000;287:215-218.
37. Liedtke W, Choe Y, Marti-Remon MA, et al. Vanilloid receptor–related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell*. 2000;103:525-535.

38. Strotman R, Hartneck C, Nunnenmacher K, et al. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nat Cell Biol*. 2000;2:695-702.
39. Ewald H, Degn B, Mors O, et al. Significant linkage between bipolar affective disorder and chromosome 12q24. *Psychiatr Genet*. 1998;8:131-140.
40. Peng JB, Chen XZ, Berger UV, et al. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J Biol Chem*. 1999;274:22739-22746.
41. Müller D, Hoenderop JGJ, Meij IC, et al. Molecular cloning, tissue distribution, and chromosomal mapping of the human epithelial Ca^{2+} channel. *Genomics*. 2000;67:48-53.
42. Montell C, Birnbaumer L, Flockerzi V, et al. A unified nomenclature for the superfamily of TRP cation channels. *Mol Cell*. 2002;9:229-231.
43. Szolcsányi J. Actions of capsaicin on sensory receptors. In: Wood JN, ed. *Capsaicin in the Study of Pain*. San Diego, CA: Academic Press; 1993:1-26.
44. Mezey E, Tóth ZS, Cortright DN, et al. Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc Natl Acad Sci U S A*. 2000;97:3655-3660.
45. Szallasi A, Di Marzo V. New perspectives on enigmatic vanilloid receptors. *Trends Neurosci*. 2000;23:491-497.
46. Sanchez JF, Krause JE, Cortright DN. The distribution and regulation of vanilloid receptor VR1 and VR1 5' splice variant RNA expression in rat. *Neurosci*. 2001;107:373-381.
47. Birder LA, Kanai AJ, de Groat WC, et al. Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. *Proc Natl Acad Sci U S A*. 2001;98:13396-13401.
48. Denda M, Fuziwara S, Inoue K, et al. Immunoreactivity of VR1 on epidermal keratinocyte of human skin. *Biochem Biophys Res Commun*. 2001;285:1250-1252.
49. Mantyh PW, Rogers SD, Honore P, et al. Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science*. 1997;278:275-279.
50. Naukkarinen A, Nickoloff BJ, Farber EM. Quantification of cutaneous sensory nerves and their substance P content in psoriasis. *J Invest Dermatol*. 1989;92:126-129.
51. Bernstein JE, Parish LC, Rapaport M, et al. Effects of topically applied capsaicin on moderate and severe psoriasis vulgaris. *J Am Acad Dermatol*. 1986;15:504-507.
52. Holzer P, Barthó L. Sensory neurons in the intestine. *In: Geppetti P, Holzer P, eds. Neurogenic Inflammation*. Boca Raton, FL: CRC Press; 1996:153-168.
53. Szolcsányi J, Barthó L. Capsaicin-sensitive afferents and their role in gastroprotection: update. *J Physiol Paris*. 2001;95:181-188.
54. Drouin E. *Helicobacter pylori*: novel therapies. *Can J Gastroenterol*. 1999;13:581-583.
55. Weaver SA, Ward SG. Phosphoinositide 3-kinases in the gut: a link between inflammation and cancer? *Trends Mol Med*. 2001;7:455-466.
56. Yoshitani SI, Tanaka T, Kohno H, et al. Chemoprevention of azoxymethane-induced rat colon carcinogenesis by dietary capsaicin and rotenone. *Int J Oncol*. 2001;19:929-939.
57. López-Carillo L, Avila HM, Dubrow R. Chili pepper consumption and gastric cancer in Mexico: a case control study. *Am J Epidemiol*. 1994;139:263-271.
58. Guo A, Vulchanova L, Wang J, et al. Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the $P2X_3$ purinoceptor, and IB4 binding sites. *Eur J Neurosci*. 1999;11:946-958.
59. Yiangou Y, Facer P, Ford A, et al. Capsaicin receptor VR1 and ATP-gated ion channel $P2X_3$ in human urinary bladder. *BJU Int*. 2001;87:774-779.
60. Cockayne DA, Hamilton SG, Zhu QM, et al. Urinary bladder hyporeflexia and reduced pain behaviour in $P2X_3$-deficient mice. *Nature*. 2000;407:1011-1015.
61. Maggi CA, Borsini F, Santicioli P, et al. Cutaneous lesions in capsaicin-treated rats: a trophic role for capsaicin-sensitive afferents? *Naunyn Schmiedebergs Arch Pharmacol*. 1987;336:538-545.
62. Andrews PLR, Bhandari P. Resiniferatoxin, an ultrapotent capsaicin analogue, has anti-emetic properties in the ferret. *Neuropharmacology*. 1993;32:799-806.
63. Nozawa Y, Nishihara K, Yamamoto A, et al. Distribution and characterization of vanilloid receptors in the rat stomach. *Neurosci Lett*. 2001;309:33-36.
64. Bíró T, Maurer M, Modarres S, et al. Characterization of functional vanilloid receptors expressed by mast cells. *Blood*. 1998;91:1332-1340.
65. Kim JC, Beckel JM, Birder LA, et al. Identification of functional vanilloid receptors in human bladder urothelial cells using a nitric oxide microsensor technique and reverse transcriptase polymerase chain reaction [abstract]. *J Urol*. 2001;165(suppl):34.
66. Kwak J, Hwang SW, Cho H, et al. Intracellular ATP increases capsaicin-activated channel activity by interacting with nucleotide-binding domains. *J Neurosci*. 2000;20:8298-8304.
67. Silva C, Avelino A, Souto-Moura C, et al. A light- and electron-microscopic histopathological study of human bladder mucosa after intravesical resiniferatoxin application. *BJU Int*. 2001;88:355-360.
68. Szallasi A, Blumberg PM. Mechanisms and therapeutic potential of vanilloids (capsaicin-like molecules). In: August TJ, Anders MV, Murad F, eds. *Advances in Pharmacology*. San Diego, CA: Academic Press; 1993:123-155.
69. Bevan S, Geppetti P. Protons: small stimulants of capsaicin-sensitive sensory nerves. *Trends Neurosci*. 1994;17:509-512.
70. Franco-Cereceda A, Liska J. Potential of calcitonin-gene related peptide in coronary cardiac disease. *Pharmacology*. 2000;16:1-8.
71. Hwang SW, Cho H, Kwak J, et al. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci U S A*. 2000;97:6155-6160.
72. Premkumar LS, Ahern GP. Induction of vanilloid receptor channel activity by protein kinase C. *Nature*. 2000;408:985-990.
73. Zygmunt PM, Petersson J, Andersson DA, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature*. 1999;400:452-457.
74. Szolcsányi J. Are endocannabinoids endogenous ligands for the VR1 capsaicin receptor? *Trends Pharmacol Sci*. 2000;21:41-42.
75. Di Marzo V, Bisogno T, De Petrocellis L. Anandamide: some like it hot. *Trends Pharmacol Sci*. 2001;22:346-349.
76. Smart D, Gunthorpe MJ, Jerman JC, et al. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (VR1). *Br J Pharmacol*. 2000;129:227-230.
77. Al-Hayani A, Wease NK, Ross RA, et al. The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. *Neuropharmacology*. 2001;41:1000-1005.
78. Tsutsumi S, Tomioka A, Sudo M, et al. Propofol activates vanilloid receptor channels expressed in human embryonic kidney 293 cells. *Neurosci Lett*. 2001;312:45-49.

79. Davis JB, Gray J, Gunthorpe MJ, et al. Vanilloid receptor-1 is essential for inflammatory hyperalgesia. *Nature*. 2000;405:183-187.
80. Robbins WR, Staats PS, Levine J, et al. Treatment of intractable pain with topical large-dose capsaicin: preliminary report. *Anesth Analg*. 1998;86:579-583.
81. Szolcsányi J. Perspectives of capsaicin-type agents in pain therapy and research. In: Parris WCV, ed. *Contemporary Issues in Pain Management*. Boston, MA: Kluwer; 1991:97-124.
82. Cruz F. Desensitization of bladder sensory fibers by intravesical capsaicin or capsaicin analogs: a new strategy for treatment of urge incontinence in patients with spinal detrusor hyperreflexia or bladder hypersensitivity disorders. *Int Urogynecol J Pelvic Floor Dysfunct*. 1998;9:214-229.
83. Chancellor MB, de Groat WC. Intravesical capsaicin and resiniferatoxin therapy: spicing up the ways to treat the overactive bladder. *J Urol*. 1999;162:3-11.
84. Ozawa H, Jung SY, Fraser MO, et al. Intravesical capsaicin therapy: a review. *J Spinal Cord Med*. 1999;22:114-118.
85. Kim DY, Chancellor MB. Intravesical neuromodulatory drugs: capsaicin and resiniferatoxin to treat the overactive bladder. *J Endourol*. 2000;14:97-103.
86. De Ridder D, Baert L. Vanilloids and the overactive bladder. *BJU Int*. 2000;86:172-180.
87. Fowler CJ. Intravesical treatment of overactive bladder. *Urology*. 2000;55:60-64.
88. Cruz F, Guimarães M, Silva C, et al. Suppression of bladder hyperreflexia by intravesical resiniferatoxin. *Lancet*. 1997;350:640-641.
89. Lazzeri M, Beneforti P, Turini D. Urodynamic effects of intravesical resiniferatoxin in humans: preliminary results in stable and unstable detrusor. *J Urol*. 1997;158:2093-2096.
90. Rivas DA, Shenot P, Stuliglowa K, et al. Intravesical resiniferatoxin (RTX) treatment of detrusor hyperreflexia: results of a randomized, double-blind, placebo-controlled, multicenter trial. *J Urol*. 1999;161(suppl):276.
91. Simone DA, Nolano M, Johnson T, et al. Intradermal injection of capsaicin in humans produces degeneration and subsequent reinnervation of epidermal nerve fibers: correlation with sensory function. *J Neurosci*. 1998;18:8947-8959.
92. Hussain IF, Fowler CJ, Landon DN. Ultrastructural changes in the composition of lamina propria nerves following intravesical capsaicin therapy [abstract]. *Eur Urol*. 1999;35(suppl 2):95.
93. Dasgupta P, Chandiramani V, Parkinson MC, et al. Treating the human bladder with capsaicin: is it safe? *Eur Urol*. 1998;33:28-31.
94. Yiangou Y, Facer P, Dyer NHC, et al. Vanilloid receptor 1 immunoreactivity in inflamed human bowel. *Lancet*. 2001;357:1338-1339.
95. Facer P, Knowles CH, Tam PKH, et al. Novel capsaicin (VR1) and purinergic ($P2X_3$) receptors in Hirschsprung's intestine. *J Pediatr Surg*. 2001;36:1679-1684.
96. Király A, Sütö G, Czimmer J, et al. Failure of capsaicin-containing red pepper sauce suspension to induce esophageal motility response in patients with Barrett's esophagus. *J Physiol Paris*. 2001;95:197-200.
97. Marciniak BH, Brown B, Peterson B, et al. Adverse consequences of capsaicin exposure in health care workers. *J Am Geriatr Soc*. 1995;43:1181-1182.
98. Reilly CA, Crouch DJ, Yost GS. Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum, and pepper spray products. *J Forensic Sci*. 2001;46:502-509.

HOLOGIC®

First and Only FDA Cleared
Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager

Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System

Click or Scan
to discover more

genius™
DIGITAL DIAGNOSTICS