Unravelling the Mystery of Capsaicin: A Tool to Understand and Treat Pain

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Abstract——A large number of pharmacological studies have used capsaicin as a tool to activate many physiological systems, with an emphasis on pain research but also including functions such as the cardiovascular system, the respiratory system, and the urinary tract. Understanding the actions of capsaicin led to the discovery its receptor, transient receptor potential (TRP) vanilloid subfamily member 1 (TRPV1), part of the superfamily of TRP receptors, sensing external events. This receptor is found on key fine sensory afferents, and so the use of capsaicin to selectively activate pain afferents has been exploited in animal studies, human psychophysics, and imaging studies. Its effects depend on the dose and route of administration and may include sensitization, desensitization, with-drawal of afferent nerve terminals, or even overt death of afferent fibers. The ability of capsaicin to generate central hypersensitivity has been valuable in understanding the consequences and mechanisms behind enhanced central processing of pain. In addition, capsaicin has been used as a therapeutic agent when applied topically, and antagonists of the TRPV1 receptor have been developed. Overall, the numerous uses for capsaicin are clear; hence, the rationale of this review is to bring together and discuss the different types of studies that exploit these actions to shed light upon capsaicin working both as a tool to understand pain but also as a treatment for chronic pain. This review will discuss the various actions of capsaicin and how it lends itself to these different purposes.

I. Introduction

Capsicum frutescens is a vegetable used daily, and the substance capsaicin is responsible for its hot and spicy flavor, sought after in gastronomy. Capsaicin and several related molecules are known by the collective name capsaicinoids, and they are produced by all plants of the genus *Capsicum*, with the exception of the bell pepper (*Capsicum annum*), which produces no capsaicin.

The naturally occurring content of capsaicinoids in spices ranges typically from 0.1 mg/g in chili pepper to 2.5 mg/g in red pepper and 60 mg/g in oleoresin red pepper (Parrish, 1996). Capsaicin and dihydrocapsaicin are the major capsaicinoids produced; however, others exist and are produced in smaller quantities. Although much of the capsaicin is found in the fruit of the plant, capsaicinoids have also been detected in the stem and leaves (Estrada et al., 2002). The Scoville Heat Unit Scale (Fig. 1) is used to classify the strength of chili peppers.

The capsaicin contents of different peppers have been determined by use of liquid chromatography and range from 0.1 to 4.25 mg/g of pepper (Parrish, 1996; Al Othman et al., 2011). Pepper varieties from *Capsicum frutescens*, *Capsicum annuum*, and *Capsicum chinense* were found to contain 0.22 to 20 mg of total capsaicinoids/g of pepper (dry weight) (Thomas et al., 1998).

Global differences in the daily consumption of capsicum spices was reported to be 2.5 g/person in India, 5 g/person in Thailand (Monsereenusorn, 1983), 15 g/person in Saudi Arabia (Al Othman et al., 2011), and 20 g/person (one chili pepper) in Mexico (López-Carrillo et al., 1994).

Aside from this key role in cuisine, a number of pharmacological and pain research studies have shown multiple effects of capsaicin in a variety of physiological systems (pain, cardiovascular, respiratory, and urinary). Although capsaicin is a widely used compound, the complexities of action at its receptor, transient receptor potential vanilloid subfamily member 1 (TRPV1[1]), are often underappreciated.

Capsaicin can produce a number of pain-related effects that depend on the dose and route of administra-

[1]Abbreviations: 5HT, 5-hydroxytryptamine (serotonin); A-784168, 3,6-dihydro-3′-(trifluoromethyl)-*N*-[4-[(trifluoromethyl)sulfonyl]phenyl]-[1(2*H*),2′-bipyridine]-4-carboxamide; A-795614, *N*-1*H*-indazol-4-yl-*N*′-(5-piperidin-1-yl-2,3-dihydro-1*H*-inden-1-yl)urea; ABT-102, (*R*)-(5-*tert*-butyl-2,3-dihydro-1*H*-inden-1-yl)-3-(1*H*-indazol-4-yl)-urea; AMG 517, *N*-(4-(6-(4-trifluoromethylphenyl)pyrimidin-4-yloxy)benzothiazol-2-yl)acetamide; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AS1928370, *N*-(1-methyl-2-oxo-1,2,3,4-tetrahydro-7-quinolyl)-2-((2-methylpyrrolidin-1-yl)methyl)biphenyl-4-carboxamide; BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; BK, bradykinin; BoNT-A, botulinum toxin serotype A; CaMKII, Ca^{2+}/calmodulin-dependent protein kinases II; CFA, complete Freund's adjuvant; CGRP, calcitonin gene-related peptide; CNS, central nervous system; CREB, cAMP response element-binding; DH, dorsal horn; DRG, dorsal root ganglion; DRR, dorsal root reflex; DTA, diphtheria toxin fragment A; EEG, electroencephalography; ENF, epidermal nerve fiber; ET, endothelin; fMRI, functional magnetic resonance imaging; HIV-AN, HIV-associated neuropathy; IBS, irritable bowel syndrome; KN-93, 2-(*N*-[2-hydroxyethyl])-*N*-(4-methoxybenzenesulfonyl)amino-*N*-(4-chlorocinnamyl)-*N*-methylamine; KO, knockout; LTP, long-term potentiation; NGF, nerve growth factor; NMDA, *N*-methyl-D-aspartate; NNT, number needed to treat; OA, osteoarthritis; OLDA, *N*-oleoyldopamine; P450, cytochrome P450; PAF, peripheral afferent fiber; PAG, periaqueductal gray; PDN, painful diabetic neuropathy; PG, prostaglandin; PHN, postherpetic neuralgia; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; Pirt, phosphoinositide-interacting regulator of TRP; PK, pharmacokinetics; PKA, protein kinase A; PKC, protein kinase C; ROS, reactive oxygen species; RTX, resiniferatoxin; RVM, rostroventral medulla; SNP, single-nucleotide polymorphism; SP, substance P; TMD, transmembrane domain; TRP, transient receptor potential; TRPA, transient receptor potential ankyrin; TRPM, transient receptor potential melastatin; TRPV, transient receptor potential vanilloid; WDR, wide dynamic range; WT, wild type.

Chili Pepper	Scoville Rating
Pure Capsaicin	15.000.000
Nordihydrocapsaicin	9.100.000
US Pepper Spray	2.000.000-5.500.000
Naga Jolokia	855.000-1.041.427
Red Savina Habenero	350.0000-577.000
Habanero Chili, Scotch Bonnet	100.000-350.000
Jamaican Hot Pepper, Rocoto	100.000-200.000
Thai Pepper, Malagueta Pepper Chiltepin Pepper, Pequin Pepper	50.000-100.000
Cayenne Pepper, Ají Pepper Tabasco Pepper	30.000-50.000
Serrano Pepper	10.000-23.000
Wax Pepper	5.000-10.000
Jalapeño Pepper	2.500-8.000
Rocotillo Pepper	1.500-2.500
Poblano Pepper	1.000-1.500
Anaheim Pepper	500-2.5000
Pimento	100-500
Bell Pepper	0

FIG. 1. The Scoville Scale. The Scoville rating indicates how "spicy" a pepper is, which depends on its respective capsaicin content. Pure capsaicin is more than 3 times hotter than pepper spray used in the United States and 200 times hotter than the average jalapeno!

tion, activation, and modulation at each. In addition, splice variants and polymorphisms identified in both animals and humans are discussed. Finally, TRPV1 expression in human peripheral and visceral tissues are explored.

We then consider the use of capsaicin in models of pain on the basis of its ability to activate pain-sensing afferents. To understand signaling between the peripheral fibers and the central nervous system, it is important to be able to assess the roles of receptors, channels, and associated molecules in the complex processes that transduce external stimuli to electrical and chemical signals. Sensory inputs from the periphery terminate in the spinal cord, where integration and hypersensitivity can be established. Spinal outputs run to limbic structures, where the affective component of pain is established and in parallel to cortical areas via the thalamus, where the coding mapping of the body on the cortex and cortical homunculus allows the location and intensity of pain to be generated. Centers of the brain important in emotional and aversive responses to pain are then recruited. These centers in the brain will be activated not only by nociceptive input but also by "top-down" processes, such as fear, anxiety, and other life events. Descending controls from the midbrain and brainstem allow the spinal cord to be regulated by descending pathways from the brain (Fig. 2).

Administration of capsaicin in animals was originally used to elucidate the function of TRPV1 as well as to aid knowledge regarding pain processing and modulation. The intraplantar injection is commonly used to study mechanisms involved in central sensitization, and these models have been developed extensively. Capsaicin-in-

tion. The consequent effects may be sensitization, desensitization, withdrawal of afferent nerve terminals, or even overt death of afferents when given to neonatal animals.

This review will first explore the physical and chemical properties of capsaicin, including its structure, pharmacology, and, importantly, pharmacokinetics. We will then give a brief overview of the TRP family ion channels, which are not only one of the largest families but also are involved in a myriad of physiological processes. From their discovery in 1969, they have been extensively studied in many laboratories to elucidate their roles and mechanisms. Here, we will focus on the TRPV1 receptor within the pain pathway, which is required for the detection of heat, protons, and of course, capsaicin. It is located in the periphery and spinal cord, in addition to some supraspinal sites. This review examines the func-

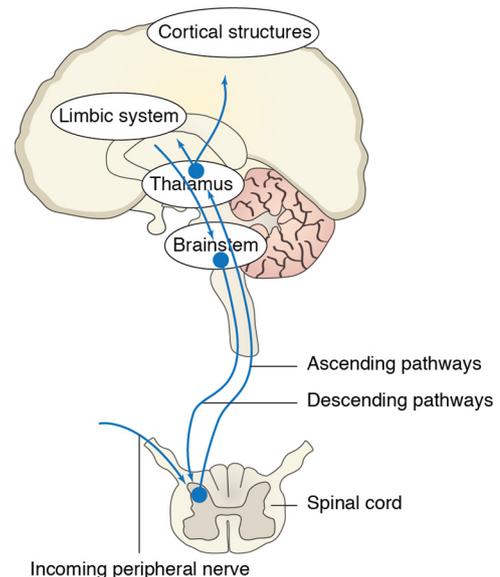FIG. 2. Pain pathways. Incoming peripheral afferent fibers input into the DH of the spinal cord. Spinal projection neurons extend and synapse in regions such as the thalamus and brainstem. From these sites, interactions are also made with the limbic system and cortical structures. Descending pathways originate in the RVM and PAG and may act on both projection neurons and afferent fibers to modulate the pain signal.
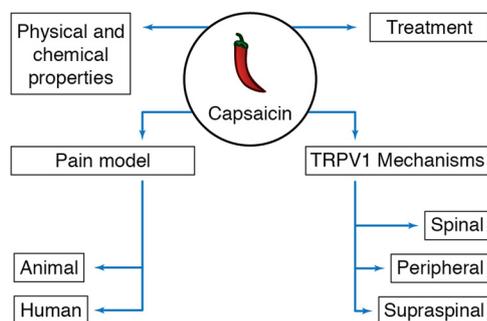
FIG. 3. Uses of capsaicin. Capsaicin is used as both an investigative tool into pain mechanisms and a treatment for chronic pain. Currently, although basic science exploits the sensitizing effects of the compound, pharmacological interventions usually rely on the desensitizing.

duced secondary hyperalgesia (a consequence of its ability to induce central sensitization—a key event in the transformation of afferent pain messages to a higher level of onward transmission via spinal cord mechanisms) has been used to investigate the roles not only of second-messenger cascades but also transmitters involved in descending modulation. The models are currently also used to assess new TRPV1 antagonists before clinical trails.

In humans, capsaicin has been used in numerous experimental pain studies, from somatic to visceral models; it can be used to assess both primary and secondary hyperalgesia. The latter is a result of central sensitization and mimics the symptoms associated with neuropathic pain, such as allodynia, secondary hyperalgesia, referred pain area, and viscerovisceral hyperalgesia. Reproducibility of different models has been investigated to verify their usefulness in unraveling mechanistic actions or testing of analgesic compounds. The models we will discuss range from intradermal capsaicin injection to topical application and oral administration.

An interesting phenomenon associated with capsaicin is its opposing effects after application. Although it is well recognized to cause pain and sensitization of both peripheral and central nerves, it can also lead to desensitization and withdrawal of epidermal nerve fibers. We consider the mechanisms behind these opposing actions, as well as how the effect is determined by method of administration, repeated application, and the dose.

It is important to recognize that the doses of capsaicin used in various studies encompass a large range. This arises from capsaicin's use as an experimental agent to activate TRPV1 in different conditions and with different aims, such as sensitization or desensitization. Because of these experimental studies, activation of TRPV1 in this manner may not be physiologically relevant. Application methods and doses used therapeutically produce only a local effect, avoiding systemic actions.

Finally, we review the uses of capsaicin not just as an investigative tool but also as a treatment for a number of neuropathic pain disorders. For example, the 8% patch is currently used in the treatment of localized neuro-

pathic conditions, such as postherpetic neuralgia (PHN). The use of an antagonist at TRPV1 is still a possibility but has yet to be used in the clinic. Here we discuss both current and possible future treatments (Fig. 3).

II. Physical and Chemical Properties of Capsaicin

Capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) is a naturally occurring alkaloid derived from plants of the genus *Capsicum*, better known as chili pepper fruit. It is a member of the vanilloid family of compounds (e.g., vanillin from vanilla, eugenol from bay leaves, cloves, and zingerone from ginger) (Hayman and Kam, 2008). Like other vanilloids, capsaicin has a benzene ring and a long hydrophobic carbon tail with a polar amide group (Fig. 4). Capsaicin is a hydrophobic, colorless, odorless, crystalline compound with the molecular formula $C_{18}H_{27}NO_3$; the melting point is 62–65°C, and the molar mass is 305.4 g/mol (Hayman and Kam, 2008; Reyes-Escogido et al., 2011). Because capsaicin is not water-soluble, alcohols and other organic solvents are used to solubilize capsaicin in topical preparations and sprays. This liposolubility is likely to explain why an oral excess of capsaicin in food is not alleviated by drinking water, whereas a yogurt-based drink, such as a lassi, is able to remove the vanilloid from the mouth.

III. Pharmacokinetics of Capsaicin

Capsaicin can be administered by number of different routes, including, in humans, exposure to the ingredient through consumption and in self-defending actions (pepper sprays), as well as topical analgesics. However, in basic science studies, the main methods are intradermal/intraplantar injections.

A. Oral Administration

Capsaicin is absorbed by a nonactive process from the stomach and whole intestine (Leelahuta et al., 1983; Kawada et al., 1984), where the total absorption capacity varies between 50 and 90% in different animal studies (Leelahuta et al., 1983; Kawada et al., 1984; Donnerer et al., 1990). Maximum blood concentration is seen 1 h after administration (Suresh and Srinivasan, 2010) (Fig. 5). To a certain extent, capsaicin is absorbed in its intact form, and the amount in the portal blood is mea-
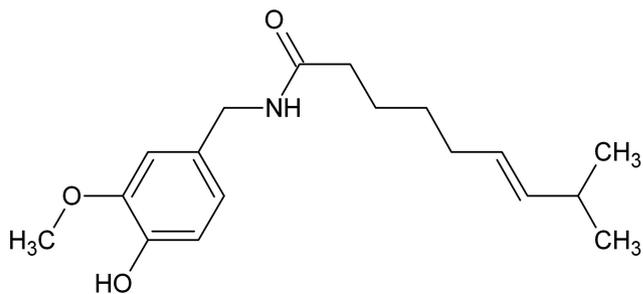

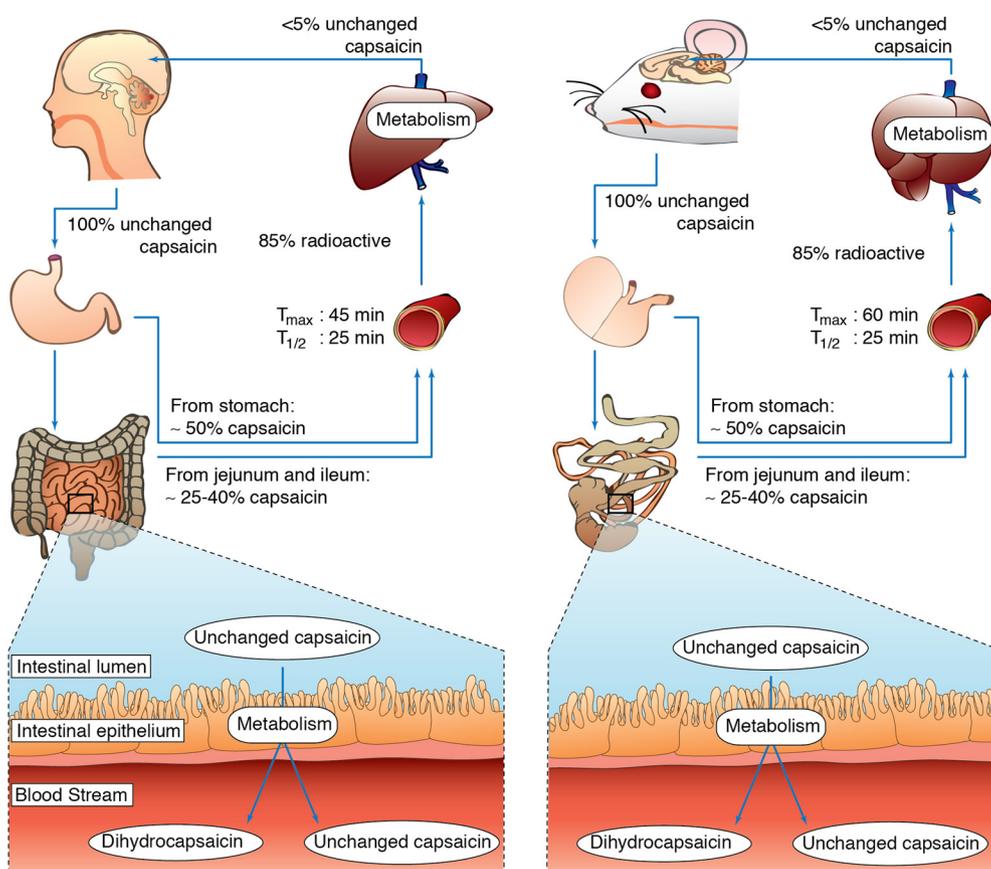FIG. 4. The molecular structure of capsaicin.

FIG. 5. Pharmacokinetics of capsaicin showing absorption, metabolism, and distribution after oral application of capsaicin in rats (left) and humans (right).

surable by labeling capsaicin with [³H]dihydrocapsaicin radioactivity (Kawada et al., 1984; Donnerer et al., 1990). However, the role of gut-epithelial absorption has been investigated in vivo by quantifying the percentage of unchanged labeled dihydrocapsaicin in male rats, showing decreased unchanged labeled dihydrocapsaicin with the route of passage of the drug (gastrointestinal lumen > portal vein blood > trunk blood and brain) (Donnerer et al., 1990). Moreover, increased numbers of intact molecules in the portal vein blood were found when absorption occurred solely from the stomach compared with absorption from stomach and small intestine, indicating metabolism of a minor part of capsaicin and dihydrocapsaicin in the small intestine epithelial cells, whereas no metabolism occurs in the intestinal lumen (Kawada et al., 1984; Donnerer et al., 1990).

The literature of orally administered capsaicin in humans is sparse. A recent study investigated thoroughly the human pharmacokinetic profile of 5 g of orally ingested *C. frutescens*, equipotent to 26.6 mg of pure capsaicin (this is the equivalent of eating 15 habanero peppers!) (Chaiyasit et al., 2009). The group documented that capsaicin could be detected in plasma after 10 min, and that peak concentration (C_{max}) was 2.47 ± 0.13 ng/ml, t_{max} was 47.1 ± 2.0 min, and $t_{1/2}$ was 24.9 ± 5.0 min (Chaiyasit et al., 2009). After 90 min, capsaicin could not be detected (Chaiyasit et al., 2009). These

results suggest that Bernard et al. (2008) were unable to create a pharmacokinetic profile of capsaicinoids after administration of 15 and 30 mg of capsaicin /person, because they used a lower detection limit of 10 ng/ml. Oral capsaicin has more relevance to molecular gastronomy than to any therapeutic options.

B. Systemic Administration

Systemic administration of capsaicin involves both intravenous and subcutaneous methods. Three minutes after intravenous injection in animals, capsaicin produces approximately 5-fold higher concentrations of unchanged capsaicin in the brain and spinal cord compared with the blood (Saria et al., 1981; Donnerer et al., 1990; Johnson, 2007). The concentration in liver was approximately 3-fold higher than that in blood. In addition, after subcutaneous injection in animals, capsaicin can be detected in spinal cord, brain, and plasma, which is illustrated schematically in Fig. 6 (Dickenson et al., 1990; Donnerer et al., 1990). There are, to the best of our knowledge, no reports of capsaicin administered intravenously in humans; given the likely widespread adverse effects, this is just as well.

C. Topical Administration

The majority of pharmacokinetic studies on capsaicin distribution are those performed after topical administra-
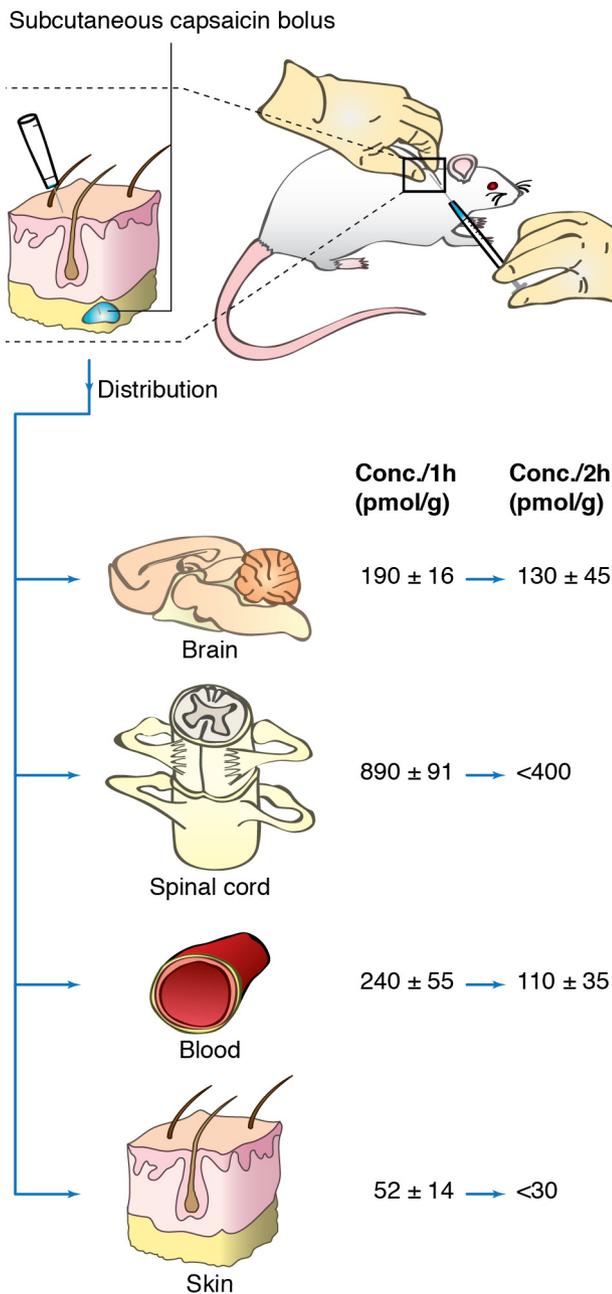
FIG. 6. The distribution of capsaicin in various tissues after subcutaneous administration in rats. The numerical values refer to concentrations after distribution to brain, blood, and skin. In the case of the spinal cord, the values refer to concentrations after local administration. Because administration is subcutaneous, the values cannot be compared with the experimental pain models in which capsaicin is administered intradermally.

tion because of the important therapeutic implications of this route. In vitro percutaneous absorption of vanillylnonamides (capsaicin analogs) in animals has been shown in dorsal skin collected in shaved hairy rats and "fuzzy" rats (Kasting et al., 1997), exemplifying the use of this route to deliver the vanilloid to peripheral neurons.

Topical capsaicin in humans is rapidly and well absorbed through the skin (Hayman and Kam, 2008), and many low concentrations of capsaicin (0.025–0.1%) are available over the counter as creams or patches. A study of

12 subjects evaluated the topical application of 3% solutions of capsaicin (55% capsaicin, 35% hydrocapsaicin, and 105 other analogs) using three different vehicle preparations (70% isopropyl alcohol, mineral oil, and propylene glycol containing 20% alcohol). Capsaicinoids were detected in the stratum corneum within 1 min after application, and a steady state was reached shortly thereafter. Maximum concentration was nearly three times greater in the subjects who received 70% isopropyl alcohol compared with the mineral oil or propylene glycol preparations. The half-life of capsaicin is approximately 24 h (Pershing et al., 2004; Hayman and Kam, 2008).

A prescribed 8% topical capsaicin patch (NGX-4010) has been introduced and labeled for pain treatment, indicated for the management of neuropathic pain associated with postherpetic neuralgia. The patch contains $640 \mu g/cm^2$, meaning that each patch contains a total of 179 mg of capsaicin (Jones et al., 2011). To determine systemic capsaicin exposure after single 60- or 90-min NGX-4010 applications, plasma samples were collected from 173 patients with PHN, painful HIV-associated neuropathy (HIV-AN), and painful diabetic neuropathy (PDN). The percentages of patients with quantifiable levels of capsaicin at any time point were 31% for PHN (30/96), 7% for HIV-AN (3/44), and 3% for PDN (1/33). The maximum plasma concentration observed in any patient was 17.8 ng/ml (Babbar et al., 2009). Because of the limited number of quantifiable levels, a population analysis was performed to characterize the pharmacokinetics (PK) of capsaicin. Plasma concentrations were fitted adequately using a one-compartment model with first-order absorption and linear elimination. Capsaicin levels declined very rapidly, with a mean population elimination half-life of 1.64 h. Mean area under the curve and C_{max} values after a 60-min application were $7.42 ng \cdot ml^{-1} \cdot h^{-1}$ and 1.86 ng/ml, respectively (Babbar et al., 2009). Correlations between calculated PK parameters and patient characteristics were observed. Duration and area of application of the patch were detected as significant covariates explaining the PK of capsaicin. Ninety-minute applications of NGX-4010 resulted in capsaicin area under the curve and C_{max} values approximately 1.78- and 2.15-fold higher than those observed in patients treated for 60 min. Treatment on the feet (patients with HIV-AN and PDN) produced far lower systemic exposure than treatment on the trunk (patients with PHN). The low systemic exposure and very rapid elimination half-life of capsaicin after NGX-4010 administration are unlikely to result in systemic effects and support the overall safety profile of this investigational cutaneous patch (Babbar et al., 2009). The medical use of topical capsaicin (which, as the above-mentioned studies show, acts almost exclusively at peripheral local sites) is discussed in further detail in section XIV.

D. Intradermal Administration

Chanda et al. (2008) investigated the in vitro metabolism of capsaicin in human skin and found that biotransformation in human skin was very slow. During incubation, capsaicin was slowly metabolized over 20 h. In addition, two metabolites were detected, vanillylamine and vanillic acid.

When capsaicin is injected intradermally in humans, it is associated with a spontaneous burning pain that subsides within few minutes (Jantsch et al., 2009). The injection leads to primary and secondary hyperalgesic areas. Sensitivity to heat has been reported to be confined to an area approximately 1 cm in diameter, centered approximately on the injection site. In addition, a much larger dose-dependent area of hyperalgesia to mechanical stimuli develops around the injection site (LaMotte et al., 1992). This study further demonstrated dose-dependent sensitivity, with lower doses leading to sensitization and higher doses to desensitization—this may account for the presence of analgesia to pinprick at the site of injection. Studies have reported a large number of nonresponders regarding the presence of hyperalgesia (Park et al., 1995; Liu et al., 1998) A number of variables could be responsible for this variation; it is therefore important to keep in mind that several factors can influence the size of the area [e.g., dose, stimulus used to test (size of von Frey hair, cotton swab, brush), time of assessment, and temperature of skin before injection]. However, the model has proved to be reliable and reproducible and has been widely used in clinical studies investigating pain and analgesia (Staahl et al., 2009a,b). In general, intradermal capsaicin is used to induce central sensitization and altered pain sensitivity, which will be discussed in section XII.

IV. Capsaicin Metabolism

Capsaicin metabolism after oral administration is believed to be similar in the human, rat, and canine microsome. When capsaicin and dihydrocapsaicin reaches the liver, a major part is metabolized; however, the proportion that undergoes metabolism is unclear. In vitro experiments show that the amount of capsaicin and dihydrocapsaicin is greatly reduced after incubation with liver homogenates (Donnerer et al., 1990; Chanda et al., 2008). In situ experiments in rats have shown that the intestinal elimination rate of capsaicin and dihydrocapsaicin is approximately equal to the concentration of radioactivity in mesenteric venous blood, indicating that presumably no metabolism take place in the gut lumen (Kawada et al., 1984).

An in vitro human investigation with hepatic microsomes and S9 fractions (used to investigate involvement of phase 2 metabolisms), showed that capsaicin was rapidly metabolized, producing three major metabolites, 16-hydroxycapsaicin, 17-hydroxycapsaicin, and 16,17-hydroxycapsaicin, whereas vanillin was a minor metabolite (Chanda et al., 2008). Moreover, Chanda et al. (2008) established a model to elucidate the metabolism of capsaicin at various concentrations physiologically equivalent to those obtained after oral ingestion of pepper fruits. They showed that capsaicin metabolism was less extensive at a concentration of 10 μM than at 1 μM (more than 50% direct inhibition of CYP1A2, CYP2C9, and CYP2C19), and the authors suggest therefore that the rate of metabolism is saturable. Although many enzymes may play some role in hepatic drug metabolism, cytochrome P450 (P450) enzymes are quantitatively the most important, and many drug-drug interactions result from the alteration (increase or decrease) in the activities of these enzymes. At the much lower plasma capsaicin concentrations occurring after topical administration, such as via the 8% patch, direct inhibition of any P450 enzyme has not been shown. Because inhibition of CYP2E1 is thought to prevent the metabolic activation of several carcinogens, and because capsaicin is believed to possess anticancer properties, it has been widely inferred that capsaicin is a CYP2E1 inhibitor. This implication, however, appears in only one publication (Reilly and Yost, 2006). There is no information on the ability of any of these compounds to modulate P450 activity and, to the best of our knowledge, the actions of the metabolites 16-hydroxycapsaicin and 17-hydroxycapsaicin are not known.

In vitro studies of capsaicin in human skin have shown slow biotransformation, and most capsaicin remains unchanged; only a small fraction is metabolized to vanillylamine and vanillyl acid (Chanda et al., 2008). This suggests that cytochrome P450 enzymes participate minimally in capsaicin transformation in skin compared with their key role in hepatic metabolism.

V. Capsaicin Elimination

Animal studies have shown that capsaicin is eliminated mainly by the kidneys, with a small untransformed proportion excreted in the feces and urine (Leelahuta et al., 1983; Kawada et al., 1984; Surh et al., 1995). It is excreted in both free form and glucuronide form. In vivo animal studies have shown that after 48 h, only a small amount (<10%) of an administered dose was found in feces (Leelahuta et al., 1983; Kawada et al., 1984).

VI. Capsaicin Pharmacology

Capsaicin depolarizes nociceptors and increases their cytosolic free Ca^{2+} concentration (Dickenson et al., 1990). The gene encoding the capsaicin receptor was isolated by using a Ca^{2+} imaging-based expression strategy (Caterina et al., 1997). A functional screening assay was used to isolate mRNA from the dorsal root ganglion (DRG) and create a cDNA library, which was divided into pools of clones and then transfected into human embryonic kidney (293) cells. Capsaicin induced changes in intracellular calcium levels were measured

within the transfected human embryonic kidney 293 cells and used to identify the vanilloid receptor (Caterina et al., 1997). The cloned receptor was designated vanilloid receptor subtype 1, or transient receptor potential vanilloid subfamily member 1, because a vanilloid moiety constitutes an essential chemical component of capsaicin. It is now well known that capsaicinoids exert their effects by activating the TRPV1 receptor (Hayman and Kam, 2008; Reyes-Escogido et al., 2011).

VII. Transient Receptor Potential Channels

TRP channels are one of the largest families of ion channels and have a wide variety of functional roles. In 1969, Cosens and Manning isolated a mutant photoreceptor from *Drosophila melanogaster* that caused the specimen to become blind upon exposure to bright light. This was the first TRP channel to be discovered; since then, 28 mammalian isoforms have been identified, which are split into seven different subfamilies (Clapham, 2003). They are made up of six transmembrane domain (TMD) polypeptide subunits that assemble as tetramers that can form pores in the cell membrane.

TRPs are one of the most extensively studied families of ion channels present in sensory neurons. The six subfamilies include TRPV, TRPC (canonical), TRPM (melastatin), TRPML (mucolipins), TRPA (ankyrin), and TRPP (polycystin) (Venkatachalam and Montell, 2007). TRPM8 and -A1 are thought to be involved in cold sensing, whereas seven others are activated by heat, over a distinct range of temperatures: TRPV1–4, TRPM2, TRPM4, and TRPM5. Collectively, these nine channels are known as thermo-TRPs and are activated at different ranges of temperature, both noxious and innocuous. TRPV2 has the highest threshold for activation (over 52°C) (Caterina et al., 1999). It has been suggested that TRPV1 and TRPA1 are the first to detect noxious hot and cold stimuli, respectively, with activation thresholds in humans of 42°C for TRPV1 and 14°C for TRPA1 (Dhaka et al., 2006); thus, activation of these receptors is proposed to lead to the perception of hot or cold thermal pain, respectively.

Another member of the TRP family, TRPM8, is believed to be responsible for the detection of cooling (30–15°C) and noxious cold (<15°C). TRPM8 may also be the receptor for menthol, which has been shown to cause cooling and eventually irritation and pain (Wasner et al., 2004). It is a nonselective cation channel, and activation generates currents required for cold sensing. TRPM8 knockout mice show deficiencies in cold detection as well as impaired development of cold hypersensitivity (Colburn et al., 2007; Dhaka et al., 2007). If TRPV1 neurons are knocked out during embryonic development, the mice also lack TRPM8-expressing neurons, suggesting that the two hot and cold receptors are colocalized during development (Mishra et al., 2011). This overview of the TRP family of channels now sets the scene for concentration of the TRPV1 receptor, the target for capsaicin.

VIII. Transient Receptor Potential Vanilloid 1

A. Introduction

The TRPV1 receptor is a nonselective ligand-gated cation channel. It is an integrator of many physical and chemical stimuli, including capsaicin and noxious heat (>43°C), as well as being activated by protons (pH <5.2), endogenous lipids, and certain inflammatory mediators (Szallasi and Blumberg, 1999). All compounds are lipophilic and therefore act at intracellular binding sites (Yang et al., 2003). Stimuli are detected and transduced through opening of the ion channel, which results in entry of cations such as Na^+ and Ca^{2+} to the neuron; although the channel is nonselective, it has been shown to have a high preference for Ca^{2+} (Caterina et al., 1997).

TRPV1 has a pore-forming hydrophobic stretch between TMDs 5 and 6 and is believed to exist as a homo- or heteromeric complex consisting of four subunits (Caterina et al., 1997; Kedei et al., 2001; Moiseenkova-Bell et al., 2008). The presence of specific amino acid residues is required for sensitivity to different stimuli; it is believed that Tyr511 and Ser512 located between TMDs 2 and 3 dictate vanilloid/capsaicin sensitivity because mutations to tyrosine or alanine render the channel capsaicin-insensitive (Jordt and Julius, 2002). As-yet unreported polymorphisms at these sites could underlie differential pain sensitivities.

There are a number of modulators of TRPV1, including certain enzymes and inflammatory mediators (Szallasi et al., 2007). Sensitization of TRPV1 is achieved through phosphorylation by certain kinases (Premkumar and Ahern, 2000; Bhave et al., 2003; Jung et al., 2004). It is believed that inflammatory mediators, discussed later, activate these enzymes through second-messenger cascades. When sensitized, the activation threshold of TRPV1 is lowered (Moriyama et al., 2005). On the other hand, phosphatases such as calcineurin and protein phosphatases 2A and 2B cause desensitization and thus an increase in activation thresholds (Zhang et al., 2006; Por et al., 2010). This is achieved via dephosphorylation in a Ca^{2+}-dependent manner (Jung et al., 2004; Mohapatra and Nau, 2005). Activity of TRPV1 can therefore be dictated by the state of phosphorylation of the channel.

TRPV1 has a number of functions and is involved in different physical processes depending on its location. One main role on peripheral nerve endings has been suggested to involve the detection of noxious heat and chemicals (Caterina et al., 1997; Jung et al., 2004), although it is also believed to play an important role in thermoregulation (Caterina, 2007). Its role in the central nervous system (CNS), although it still involves

pain processing and modulation, is currently less well understood.

The receptor also seems to play an important role in certain chronic pain conditions such as neuropathic pain, osteoarthritis (OA), bone cancer pain, inflammatory bowl disease (IBD) and migraine (Szallasi et al., 2007; Alawi and Keeble, 2010). In these conditions mediators such as ATP and nerve growth factor (NGF) are capable of augmenting TRPV1 activity (Schumacher, 2010). Most effects occur in the periphery at the site of inflammation, which will be discussed in further detail in section C.

B. Transient Receptor Potential Vanilloid 1-Associated Molecules

In addition to phosphorylation of the channel, TRPV1 is reported to associate with a number of intracellular proteins that may also modulate receptor activity and trafficking to the membrane—and thus affect function of the channel. Reported interactions include phosphatidylinositol 4,5-bisphosphate (PIP$_2$), phosphoinositide-interacting regulator of TRP (Pirt), GABA-receptor-associated protein, soluble N-ethylmaleimide-sensitive factor attachment protein receptor, A-kinase anchoring protein 150, and components of the cytoskeleton. Here we will focus on PIP$_2$ and the cytoskeleton.

1. Phosphatidylinositol 4,5-Bisphosphate. PIP$_2$ is a membrane phospholipid required for a number of intracellular signaling pathways. It has been shown to be associated with TRPV1 in the plasma membrane, through an interaction at the C-terminal (Prescott and Julius, 2003; Ufret-Vincenty et al., 2011). Two opposing actions have been proposed with regard to how PIP$_2$ regulates TRPV1 function. Although it was originally believed that binding of PIP$_2$ to TRPV1 was inhibitory, later studies suggested the contrary. Prescott and Julius (2003) reported that a mutation in the C-terminal of TRPV1, inhibiting the binding of PIP$_2$, resulted in larger capsaicin currents. However, this finding relied on an absence of extracellular Ca^{2+}, and further in vitro and in vivo studies have produced opposing findings. Stein at al demonstrated that polylysine (an agent that sequesters PIP$_2$) had an inhibitory effect on TRPV1, whereas Liu et al. (1998) found that replenishing PIP$_2$ aided recovery after desensitization. More recently, Sowa et al. (2010) demonstrated in a number of in vivo experiments that PIP$_2$ was required both for normal sensing of noxious heat and for the development of sensitization. PIP$_2$ enhanced thermosensation for up to 2 h and increased thermal hypersensitivity and mechanical allodynia in models of inflammation and nerve injury. Taken together, this evidence suggests there is an overriding pronociceptive role of PIP$_2$.

It has been suggested that imperative for this interaction with PIP$_2$ is the regulatory subunit Pirt. It has been shown that the C-terminal of Pirt binds to both TRPV1 and PIP$_2$ and that Pirt is required for enhancing TRPV1 signaling through PIP$_2$ (Kim et al., 2008). In addition, Pirt($-$/$-$) mice were found to have impaired response to heat and capsaicin. This suggests that association with Pirt may also be required for optimum functioning of TRPV1. However, this has not been reproduced in subsequent studies, and it has been suggested that the phosphoinositide sensitivity of TRPV1 is not altered by Pirt (Ufret-Vincenty et al., 2011). Further investigation into the small molecules that associate with TRPV1 and may modulate activity will be of great interest. Novel modulation of this channel, targeting associated molecules, could be useful when designing drugs to alter the function of TRPV1 but keep side effects minimal.

2. Cytoskeleton. Recent evidence suggests that the cytoskeleton is not just important for structural integrity but may also play a role in signal transduction and transmission of nociceptive information between neurons (Bhave et al., 2003). A number of studies have further shown that TRPV1 forms a signaling complex with elements of the cytoskeleton, allowing the integration of signals and exchange of synaptic information. This suggests that TRPV1 is important for structural and functional regulation as well as for neuronal communication and network formation, in addition to the pivotal role already discussed in the detection of noxious stimuli and transduction of pain signaling (Goswami, 2010). Through a number of investigations, Goswami et al. (2004, 2006) have demonstrated that TRPV1 interacts with tubulin, in particular polymerized dynamic microtubules. Microtubules are made up of polymers of tubulin, which are integral for the maintenance of cell structure and intracellular transport. Activation of TRPV1 may therefore lead to cytoskeletal reorganization, such as rapid disassembly of dynamic microtubules, which may be involved in the detraction of sensory afferents (Goswami, 2010). In addition, they found that TRPV1 was often localized with growth cones and filopodia tips and suggested that it may regulate the morphology and function of these structures and thus play a role in neuronal communication (Goswami, 2010). Although interaction with microtubules is believed to arise from the C terminus of TRPV1, the N terminus is thought to be important for regulation filopodial dynamics. It remains to be determined whether these interactions have functional importance in vivo in terms of sensory function.

C. In the Periphery

TRPV1 receptors are mainly expressed on primary sensory neurons. They have been detected on terminals of small- to medium-diameter nociceptors, such as peptidergic and nonpeptidergic C fibers, as well as some Aδ fibers (Caterina et al., 1997; Tominaga et al., 1998). These fibers generally project into the superficial layers of the dorsal horn (DH) including laminae I and II (To-

minaga et al., 1998). Projections may also extend into laminae V and X (Tominaga et al., 1998).

As discussed, TRPV1 is a polymodal signal transducer, imperative for the detection of capsaicin and particularly important for detection of noxious heat. Activation of receptors causes depolarization of nociceptors (Bevan and Szolcsányi, 1990), which allows the pain signal to be transduced and relayed through the spinal cord to the brain, where it is processed and perceived. Caterina et al. (2000) demonstrated that capsaicin sensitivity was eliminated in TRPV1($-/-$) mice; interestingly, however, they had only impaired heat detection and reduced thermal hypersensitivity in response to inflammatory agents. This also highlights the importance of TRPV1 for the induction of thermal hyperalgesia in inflammatory states, but incomplete loss suggests there may be additional channels involved in normal heat sensing or compensatory changes.

However, more recently, the function of TRPV1 positive afferents has further been elucidated with the profiling of TRPV1-DTA mice generated by Mishra et al. (2011). TRPV1-Cre animals were crossed with a ROSA-stop-diphtheria toxin fragment A (DTA) line to ablate a specific population of TRPV1-expressing fibers. This enables the study of the function of the neuronal population rather than just TPV1 itself. As noted, it was shown previously that TRPV1 KO mice maintained some thermosensation; it was impaired only over 50°C (Caterina et al., 2000). However, these mice, whose TRPV1 afferents are completely ablated and have no response to capsaicin, are also totally insensitive to both hot and cold (Mishra et al., 2011). The mice also exhibited no hypothermia in response to intraplantar capsaicin, which is seen in normal animals. This suggests that TRPV1-positive afferents may be more imperative in the thermoregulation and detection of noxious heat than was previously thought.

Thermal hyperalgesia develops in certain pathological states in which peripheral TRPV1 may be sensitized by a number of mediators acting through intracellular signaling pathways (Kanai et al., 2007). For example, protons are able to both directly activate and potentiate activity of TRPV1. During a state of tissue injury or ischemia, when proton levels may be elevated, hydrogen ions are thought to act at an extracellular site to increase the potential of channel opening (Jordt et al., 2000; Huang et al., 2006). On the other hand, mediators such as prostaglandins, including PGE_2 and PGI_2, act at EP_1 or IP receptors, respectively, which are coupled to G_s. They have been demonstrated to interact with TRPV1 through PKA-dependent pathways, resulting in lowering of the temperature activation threshold to as low as 35°C (Smith et al., 2000; Moriyama et al., 2005). Other mediators, such as bradykinin (BK), ATP, and endothelin (ET)-1 act at G_q-coupled receptors— B1/B2, $P2Y_2$ and ET_A, respectively. This is believed to activate the diacylglycerol-PKC pathway (Vellani et al., 2004),

therefore once again resulting in phosphorylation of TRPV1. PKCε has been implicated in phosphorylation of TRPV1 at serine residues 502 and 800 because cells containing the mutations S502A or S800A are unable to sensitize (Numazaki et al., 2002; Kawamata et al., 2008). Finally, an influx of Ca^{2+} through TRPV1 itself and release from intracellular stores can result in the activation of CaMKII (Fig. 7).

This aforementioned sensitization, intriguingly, could allow the receptor to become active at body temperature, so it not only contributes toward thermal hypersensitivity but also is possibly a substrate for ongoing burning pain that patients often report. It has been suggested that the pain that is felt from mediators, such as BK alone, may be as a result of this interaction with TRPV1—suggesting that in addition to the traditional view of TRPV1 directly gating nociceptive signals, it may also indirectly gate other signals through intracellular signaling pathways (McMahon and Wood, 2006). This would involve the binding of BK to B1/B2 receptors and initiation of PKC-dependent signaling pathways, resulting in phosphorylation of TRPV1 and a reduction in heat pain threshold to body temperature, and thus an "ongoing" pain is felt.
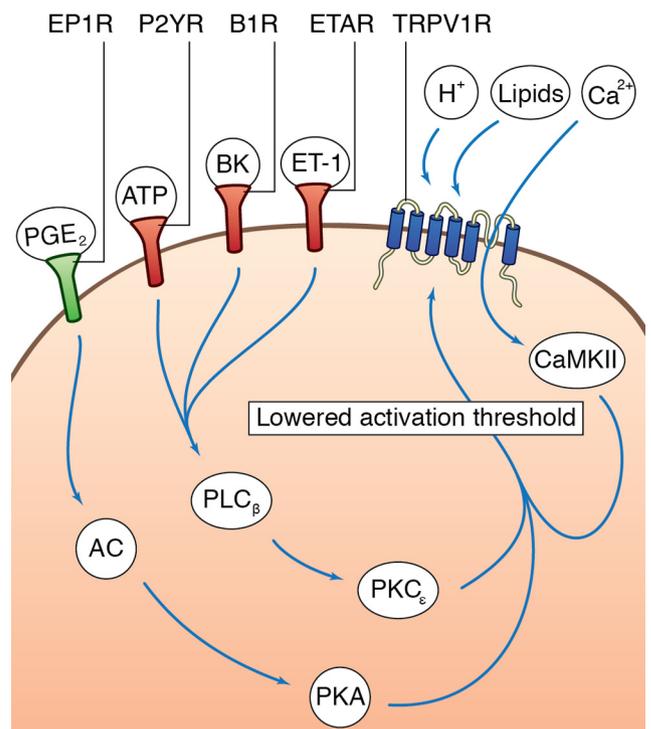
FIG. 7. Phosphorylation of peripheral TRPV1. There are a number of endogenous inflammatory mediators, such as PGE_2, ATP, BK, and ET-1, which are able to act at their respective receptors located on TRPV1-expressing afferent fibers. Through various intracellular signaling cascades, they are able to phosphorylate, and sensitize, TRPV1 (although certain lipids and acid may act directly on TRPV1 itself)—resulting in the lowering of activation thresholds and heightened activity to further stimuli. Furthermore, this effect may also be achieved through Ca^{2+}-dependent activation of CaMKII, which also phosphorylates TRPV1. This phosphorylation may lead to both thermal and mechanical hypersensitivity at this site. AC, adenylyl cyclase; PLC13, phospholipase C13. (See section VIII.C for details.)

The theory that TRPV1 integrates various signals from inflammatory molecules that are not direct agonists of the channel is supported by the finding that BK responses can be modulated by capsazepine and are also depleted in TRPV1 KO mice (Huang et al., 2006). Furthermore, in a study regarding PIP_2 modulation of TRPV1, the authors found that, in addition to the expected decrease in activity of TRPV1 itself, there was also a reduction in response to both BK and ATP and an inhibition of ATP-mediated thermal hyperalgesia (Sowa et al., 2010). The authors suggest that this reduction in response may be a direct effect of B1/2 and $P2Y_2$ requiring PIP_2; however, it is also plausible that it is due to attenuation of the proposed interaction with TRPV1. This would strongly suggest that BK currents are initiated through TRPV1; therefore, a role for TRPV1 in ongoing pain is not unreasonable to suggest.

A second method of potentiating the actions of TRPV1, rather than phosphorylation, is by increasing the surface expression of receptors, either through an increase in transport or in number of receptors produced. It has been suggested that in inflammatory conditions such as OA, NGF is released and contributes toward pain through actions at TrkA receptors, which are expressed on specific sensory neurons such as C and Aδ fibers (McMahon et al., 1991). Injection of complete Freund's adjuvant (CFA) and the iodoacetate model of OA have been shown to result in increased TRPV1 expression in DRG (Ji et al., 2002; Fernihough et al., 2005). Zhang et al. (2005) demonstrated that TrkA induced activation of PI3 kinase/Src kinase, causing phosphorylation of the Tyr200 residue, which resulted in increased membrane expression of TRPV1. In addition, Xue et al. (2007)Ji et al. (2002) found that it could also induce translation via p38 mitogen-activated protein kinase activation. Considering these possible roles in sensitization and potential contribution to chronic pain states, it can be inferred that TRPV1 may be an important drug target.

D. In the Viscera

Weller et al. (2011) found evidence for functional expression in viscera of excitatory TRPV1, TRPA1, and inhibitory cannabinoid 1 receptors along the sensory fibers of the vagus nerve. This finding has pathophysiological relevance to the axonal membrane and the control of neuropeptide release that may become important in cases of inflammation or neuropathy. Sensitization and possible ectopic discharge may contribute to the development of autonomic dysregulation in visceral tissues that are innervated by the vagus nerve (Weller et al., 2011).

E. In the Spinal Cord

TRPV1 is found not only on peripheral nerve endings but also in the spinal cord. Expression seems to be mainly restricted to the central branches of small and medium-sized fibers located in the dorsal root ganglia

and in the superficial DH (Szallasi et al., 1994; Guo et al., 1999). Both Valtschanoff et al. (2001) and Doly et al. (2004a) detected TRPV1 on postsynaptic second-order neurons in the rat DH. In the spinal cord, TRPV1 is believed to play a role in both the modulation and transmission of pain.

Because the spinal cord is not subject to the same changes in temperature and pH that occur in the periphery to activate TRPV1, it was assumed that endogenous agonists must be present within the CNS. Studies have highlighted several possible substances (Fig. 8), including anandamide (Zygmunt et al., 1999), metabolites of lipoxygenases (Hwang et al., 2000), ω-3 polyunsaturated fatty acids (Matta et al., 2007), N-arachidonoyldopamine (Huang et al., 2002), and N-oleoyldopamine (OLDA) (Spicarova and Palecek, 2009). Application of high concentrations of OLDA to superficial DH neurons in rat spinal cord slices increased miniature excitatory postsynaptic currents, which in turn were blocked by the addition of TRPV1 antagonists (Spicarova and Palecek, 2009). Activation of presynaptic TRPV1 receptors in the spinal cord was originally thought to result in excitation of central fibers via increased release of glutamate and other excitatory neuropeptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP). This was shown by Ueda et al. (1994), who demonstrated that application of capsaicin to slices of rat DH evoked release of glutamate and depleted stores of SP. In addition, electrophysiological studies showed that capsaicin application to DH slices from adult rats resulted in increased miniature excitatory postsynaptic currents that could be blocked by application of the TRPV1 competitive antagonist capsazepine (Yang et al., 1998).

However, spinal intrathecal administration of capsaicin results in a rapid attenuation of C-fiber input when
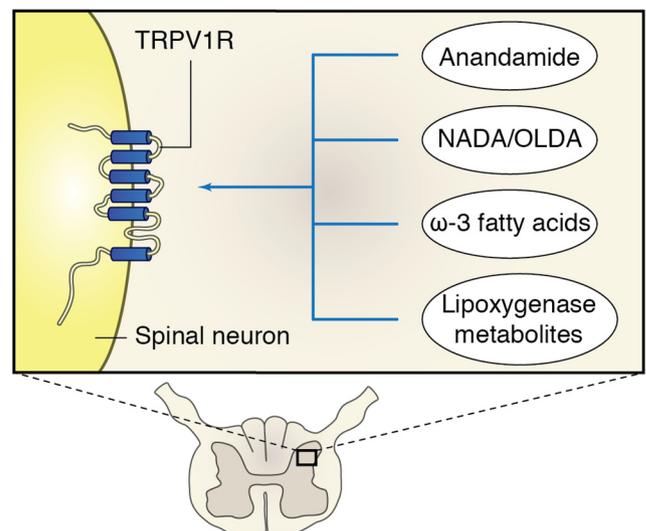

FIG. 8. Spinal activators TRPV1. The endogenous agonists of spinal TRPV1 remain unclear; however, a number of possible substances have been suggested, including anandamide, metabolites of lipoxygenases, ω-3 polyunsaturated fatty acids, N-arachidonoyldopamine (NADA), and N-oleoyldopamine (OLDA).

recording from wide dynamic range (WDR) cells. They suggest that systemic capsaicin, which had previously been shown to be antinociceptive in behavioral tests, could selectively attenuate C-fiber inputs to the DH (Dickenson et al., 1990). They concluded that the effect was unlikely to be the result of an inactivation of C-fibers at the peripheral terminals and was more likely to be the result of the actions directly on the spinal cord; SP or other substances released from primary afferent nerve terminals play a prominent role in such desensitization. Thus suggesting activation of receptors directly in the spinal cord has an inhibitory effect on the nociceptive pathway.

Ferrini et al. (2007) showed that administration of capsaicin to lamina II neuron slices from mice pups resulted in an increase of spontaneous inhibitory postsynaptic currents. The authors postulated that GABAergic inhibitory interneurons in laminae I, III, and IV are excited because of the release of SP, which in turn reduces activity in lamina II neurons. Thus provides further evidence that there is an inhibitory, as well as excitatory, role for TRPV1 in the spinal cord.

The distribution and therefore the role of spinal TRPV1 is believed to alter during pathological pain states. Dom-Bourian et al. (2006) showed that there was an upregulation of TRPV1 in the DH of rats after spinal cord injury, and Tohda et al. (2001) demonstrated that carrageenan-induced inflammation resulted in an increased transport of TRPV1 mRNA to neuron terminals in the DH. Both models were associated with thermal hyperalgesia, which has been shown to be blocked by intrathecal administration of TRPV1 antagonists such as 3,6-dihydro-3′-(trifluoromethyl)-N-[4-[(trifluoromethyl)sulfonyl]phenyl]-[1(2H),2′-bipyridine]-4-carboxamide (A-784168) and N-1H-indazol-4-yl-N'-(5-piperidin-1-yl-2,3-dihydro-1H-inden-1-yl)urea (A-795614) (Wang and Woolf, 2005; Cui et al., 2006; Yu et al., 2008). Studies have also shown that spinal TRPV1 is likely to be involved in the development of mechanical hyperalgesia (Kanai et al., 2007). Intrathecal injection of antisense oligonucleotides against TRPV1, in a rat spinal nerve ligation model, resulted in decreased mechanical hypersensitivity (Christoph et al., 2007). The results therefore strongly suggest that activation of TRPV1 has an over-riding pronociceptive effect in chronic pain states.

In contrast, the cannabinoid receptor ligand anandamide, which has also been shown to activate TRPV1, has an antinociceptive effect (which was reduced by capsazepine) when administered intrathecally in a rat model of carrageenan-induced inflammation (Horvath et al., 2008). This effect is most likely to occur through TRPV1 activation of spinal inhibitory interneurons, in combination with activation of cannabinoid receptors, resulting in decreased pain. Thus, pharmacological evidence also suggests there are two opposing actions of TRPV1 in the spinal cord.

F. Supraspinal

In addition to expression in the periphery and in the DH, TRPV1 receptors have also been detected at numerous supraspinal sites within the CNS, where it is believed to be involved in pain processing. Locations include the rostroventral medulla (RVM), periaqueductal gray (PAG), and amygdala (Millan, 2002; Liapi and Wood, 2005), as well as the nucleus tractus solitarius (Doyle et al., 2002), the somatosensory cortex, the anterior cingulated cortex, and the insula (Millan, 2002). It is important to note that endogenous agonists of supraspinal TRPV1 are unknown, and additional roles (besides the potential in pain processing) are largely unclear. Capsaicin has not been used in many studies to study supraspinal TRPV1, and further elucidation of its role will be important, including information on the endogenous activators of the channel within the CNS.

Compared with peripheral and spinal TRPV1, expression of supraspinal receptors was thought to exclusively play an analgesic role. A microinjection of capsaicin within the ventrolateral PAG was demonstrated to have an antinociceptive effect in rats (Palazzo et al., 2002; Starowicz et al., 2007). This suggests that the analgesic properties are due to an interaction with descending modulatory pain pathways.

Starowicz et al. (2007) also found that the capsaicin injection increased thermal pain thresholds. This appeared to be the result of an increase in glutamate release from neurons in the PAG, which activated mGlu and NMDA receptors, causing an eventual downstream increase in OFF cell activity in the RVM. Injection of a TRPV1 competitive antagonist, iodo-resiniferatoxin (RTX), to the ventrolateral PAG was also associated with an increase in ON cell activity (Starowicz et al., 2007), suggesting that TRPV1 activation in the PAG does lead to an increase in descending inhibition of pronociceptive pathways.

On the other hand, it has been previously demonstrated that capsaicin injection into the dorsolateral PAG resulted in hyperalgesia before analgesia set in; the authors therefore suggested that capsaicin-induced analgesia may be due to TRPV1 desensitization (McGaraughty et al., 2003). At present, the more important mechanism of supraspinal TRPV1-mediated antinociception is unclear, but much focus has been placed on the role of its activation of descending inhibitory pathways.

G. Non-Neuronal

It has also been found that a number of non-neuronal cells express TRPV1, including Schwann cells, astrocytes, and mast cells (Doly et al., 2004b; Ständer et al., 2004). It has been suggested that whereas astrocytic expression seen in the rat DH may be involved in pain modulation and central sensitization, activation of TRPV1 on the surface of mast cells may contribute to the inflammatory response through enhanced production of

interleukin-4 (Doly et al., 2004b; Ständer et al., 2004). Further discussion of non-neuronal TRPV1 expression and its contribution to pain signaling is outside the scope of this review.

IX. Transient Receptor Potential Vanilloid 1 Splice Variants

Splice variants of TRPV1 have been identified in both rats and humans. In rodents, there are three main splice variants: TRPV1 5′, TRPV1$_{VAR}$, and TRPV1β (Szallasi et al., 2007). TRPV1 5′ is missing the first 0.5 kilobases, which is the N-terminal region of wild-type (WT) TRPV1 and was shown by Sanchez et al. (2001) to be expressed in the rat DRG and the CNS. In the DRG, its expression is reported to be 12 times lower than the WT, although in the CNS levels are similar for both variants (Sanchez et al., 2001). TRPV1 5′ does not form a functioning ion channel and thus is nonresponsive to vanilloid agonists (Jara-Oseguera et al., 2008). TRPV1$_{VAR}$ is also nonfunctional, unless expressed with TRPV1 WT, where it seems to act as a negative regulator (Tian et al., 2006). TRPV1β is also believed to be a negative modulator of TRPV1 WT, despite also responding to capsaicin alone (Wang et al., 2004). In humans the splice variant TRPV1b, which is found in the DRG and CNS, lacks exon 7, and once again has been demonstrated to act as an endogenous negative regulator of TRPV1 (Lu et al., 2005; Vos et al., 2006). Vos et al. (2006) found that TRPV1b is expressed on the cell surface and forms a complex with TRPV1 to inhibit activity. The physiological relevance of TRPV1b, with regard to pain, remains unknown. However, it would be interesting to examine a possible down-regulation during chronic pain states.

It is interesting to note that it has recently been shown that alternative splicing of the TRPV1 gene in the trigeminal ganglia of vampire bats, to create the isoform TRPV1-S, can lower the activation threshold to 30°C (Gracheva et al., 2011). This ganglion specific splicing allows a modification of TRPV1 function so that the species can use it to detect infrared radiation given off by warm-blooded prey, yet the bats otherwise maintain normal function of TRPV1 in somatic afferent fibers. Thus highlighting the importance of splice variants, with regard to channel function.

X. Transient Receptor Potential Vanilloid 1 Polymorphisms

There are at least six nonsynonymous polymorphisms of the human *TRPV1* gene, though relatively little is known about their consequences—particularly with regard to pain (Xu et al., 2007). Single-nucleotide polymorphisms (SNPs) include: TRPV1^{I585V}, TRPV1^{T505A}, TRPV1^{T469I}, TRPV1^{I315M}, TRPV1^{P91S}, and TRPV1^{K2N} (Xu et al., 2007). Although most seem to have little functional significance, studies have shown that

TRPV1^{I585V}, TRPV1^{I315M}, and TRPV1^{P91S} may affect receptor activity (Kim et al., 2004; Cantero-Recasens et al., 2010). TRPV1^{I585V} involves the substitution of isoleucine for valine at position 585 in exon 11 and thus affects a TMD (Xu et al., 2007). Kim et al. (2004) demonstrated that people carrying the mutation had longer withdrawal times to cold pain. This discovery was unexpected because TRPV1 is mainly believed to be involved in the detection of heat and therefore suggests that different TRP channels may interact during temperature sensing. An increased withdrawal time would suggest the SNP results in a damping of receptor function. This is yet to be shown animal models of pain; however, investigation of the role of TRPV1^{I585V} in childhood asthma has begun to elucidate the mechanism. A study by Cantero-Recasens et al. (2010) found that the substitution for valine resulted in decreased channel activity/ Ca^{2+} entry in response to both capsaicin and heat, resulting in a 20 to 30% loss of channel function (this was associated with a lower risk of active asthma/coughing). It is plausible that this decreased activity of TRPV1 could explain people with higher heat pain thresholds and why certain subjects seem unable to develop a strong central sensitization and mechanical hyperalgesia in response to intradermal capsaicin injection, despite still perceiving the initial pain and developing thermal hyperalgesia.

In a separate study Xu et al. (2007) found that the SNPs TRPV1^{I315M} and TRPV1^{P91S} resulted in greater surface expression of the receptors. TRPV1^{I315M} and TRPV1^{P91S} SNPs effect exons 5 and 1, respectively; because TRPV1^{I315M} is in a region that is believed to encode protein binding, this may effect ligand interactions and thus agonist responses. Both TRPV1^{I315M} and TRPV1^{P91S} were demonstrated had a slight increase in maximum response to capsaicin and anandamide (Xu et al., 2007), thus suggesting that this mutation results in a gain of function of the receptor and may render carriers more sensitive to painful stimuli that are transduced by TRPV1.

A study from the German Research Network on Neuropathic Pain, assessing the impact of 11 select TRP SNPs in healthy volunteers and patients with neuropathic pain, has shown that the TRPV1 mutation 1911A>G (TRPV1^{I585V}) was associated with a decrease in heat hyperalgesia, pinprick hyperalgesia, and mechanical hypoesthesia in the patients with neuropathic pain. In addition, TRPV1^{I315M} was associated with cold hypoesthesia (Binder et al., 2011). Although they found no evidence that TRP genetic variants are associated specifically with the prevalence of neuropathic pain, the polymorphisms contributed significantly to the somatosensory abnormalities that are experienced by patients with neuropathic pain (Binder et al., 2011). Further investigation into TRPV1 SNPs and neuropathic pain conditions may be useful, but these very recent studies show that variations in TRPV1 can explain some of the

variability in pain seen in patients with pathophysiological conditions.

XI. Transient Receptor Potential Vanilloid 1 Receptor Expression in Humans in the Airways, Skin, and Viscera

Outside of the CNS, TRPV1-expressing afferents have been reported to be present in different human tissues including vascular smooth muscle, bronchial epithelial cells (Seki et al., 2007), the epidermis (Lee et al., 2009), and the gastrointestinal tract (Yiangou et al., 2001; Chan et al., 2003; Matthews et al., 2004). This suggests other roles for TRPV1 besides nociception (for a full review on the subject, see Fernandes et al., 2012).

A. Airways

It is well know that TRPV1 is expressed in the airways, on vagal and nonmyelinated C-fiber afferents (Ho et al., 2001), and it is believed to be involved in both airway constriction and cough. Lundberg and Saria (1982) found that stimulation of capsaicin-sensitive neurons resulted in smooth muscle contraction, which was confirmed by Forsberg et al. (1988) who additionally highlighted the role in cough. It was further demonstrated that TRPV1 antagonists can suppress cough, therefore confirming its pivotal role in this reflex (Trevisani et al., 2004). Recent studies have also reported potential up-regulation of TRPV1 in patients with chronic cough, suggesting that increased activity would underlie the hypersensitivity leading to this condition (Mitchell et al., 2005).

B. Skin

Studies suggest that in the epidermis, heat- and UV-induced matrix metalloproteinases-1 expression may be partly mediated by TRPV1 activation in human keratinocytes (Lee et al., 2012) and showed that TRPV1 protein was expressed at higher levels in the sun-protected skin of older subjects than in that of young people. However, photoaged skin of older subjects showed increased expression of TRPV1 mRNA and protein compared with that of the sun-protected skin of the same people. Therefore, the expression of TRPV1 is affected by both the intrinsic aging and photoaging processes (Lee et al., 2009). The increased TRPV1 expression in skin from older people implies that TRPV1 may be related to senile skin symptoms, such as senile pruritus and neurogenic inflammation; therefore TRPV1 has been proposed as an intriguing novel target for the prevention of skin aging and inflammation.

C. Gastrointestinal Tract

TRPV1-immunoreactive nerves were found to be distributed within the lamina propria from esophageal mucosal biopsies in healthy subjects and in patients with esophagitis. The percentage of papillae positive for TRPV1 was elevated in patients with esophagitis compared with control subjects (Matthews et al., 2004). Supporting this, TRPV1 has been localized in the human colon innervations, where TRPV1 immunoreactivity is greatly increased in colonic nerve fibers of patients with active IBD than in healthy bowel (Yiangou et al., 2001). The increase might be mediated by NGF, which, as discussed previously, regulates the capsaicin sensitivity of human sensory neurons and is itself increased in inflamed tissues, such as in IBD (Yiangou et al., 2001). Chan et al. (2003) compared full-thickness rectal biopsy samples from nine patients with physiologically characterized rectal hypersensitivity with tissue samples from 12 control subjects. In rectal hypersensitivity, nerve fibers immunoreactive to TRPV1 were increased in muscle, submucosal, and mucosal layers. The increase in TRVP1 correlated significantly with decrease in rectal heat and distension sensory thresholds, therefore suggesting that TRPV1 may be important in certain states of visceral inflammation.

XII. Experimental Pain Models

A. Animals

The use of capsaicin in animal models is important in the study of both processing and modulation of pain signals. As mentioned previously, capsaicin was originally used in animal models to elucidate the function of the TRPV1 receptor and its interactions in the periphery. Cloning of the capsaicin receptor by Caterina et al. (1997) was followed by in vivo analysis of its mechanisms using an intradermal capsaicin injection into the plantar skin of the hind paw in mice. It was initially given to wild-type animals before being administered to TRPV1(−/−) variants and DTA KOs, which highlighted the importance of the receptor in detection of capsaicin itself, as well as noxious heat (Caterina et al., 1997). The development of thermal and mechanical hyperalgesia was also impaired, highlighting the role of TRPV1 in the development of these symptoms (Caterina et al., 1997).

Although thermal hypersensitivity is regarded as a peripheral phenomenon discussed previously, sensitization to mechanical stimulation is thought to be underpinned by central mechanisms such as an increased excitability of DH neurons. Ongoing stimulation of peripheral C and Aδ fibers—for example, because of capsaicin injection or large areas of topical application—can result in wind-up, central sensitization, and long-term potentiation (LTP). The increased input into the DH causes an increase in release of glutamate and neuropeptides, such as SP and CGRP, at the central synaptic terminal. This in turn results in an increased activation of postsynaptic receptors and a slow depolarization of second-order projection neurons, thus relieving the usual block of NMDA receptors (Woolf and Thompson, 1991). Once NMDA receptors are also activated, changes may occur within the second-order projection neurons,

including an increased surface expression of postsynaptic receptors. This may be due to a number of mechanisms involving changes in transcription and translation, as well as post-translational modifications (Fig. 9). These changes are believed to be responsible for the symptoms associated with secondary hyperalgesia in both humans and animals. Although wind-up is only a short-term phenomenon and is reversible, central sensitization and LTP can induce long-term changes.

Before the discovery of the receptor, intradermal injection of capsaicin was shown to produce secondary hypersensitivity to noxious and innocuous stimuli in both monkeys and rats (Baumann et al., 1991; Sluka and Willis, 1997). As mentioned, these symptoms (which in humans are known as secondary hyperalgesia and allodynia) are markers of a state known as central sensitization, where DH neurons have become hyperexcitable and elicit greater responses than usual. It is associated with many chronic inflammatory and neuropathic pain states and therefore is imperative that mechanisms are understood. Dougherty and Willis (1992) gave intradermal capsaicin to monkeys and showed 1) that discharge rate of second-order neurons in the DH increased after injection and 2) an increase in release of the excitatory amino acids glutamate and aspartate. They also found an increase in activation of NK1 receptors on second-order neurons, which, they hypothesized, might be contributing to the sensitization (Dougherty et al., 1994).

Subsequently, a rodent model was used, and once again it was shown that intradermal injection of capsaicin could lead to increased response of second-order neurons (Fig. 10); this was associated with an increased release of neurotransmitters in the DH such as glutamate and SP (Willis, 1997; Yan et al., 2006). Willis, 2001 demonstrated that a heightened release of neurotransmitters and neuropeptides in response to capsaicin resulted in an increased activation of AMPA, NMDA, mGlu and NK1 receptors on second-order neurons. It was later shown that SP synthesis, as well as release, was amplified after injection and is also believed to contribute to sensitization of second-order neurons (Yan et al., 2005).

Sun et al. (2003) have since shown that CGRP is another important neuropeptide contributing to central sensitization. TRPV1 is expressed on peripheral peptidergic C fibers, some of which contain CGRP—a peptide that is believed to be released in the DH after a prolonged activation of the receptor. Administration of the CGRP receptor antagonist $CGRP_{8-37}$ into the DH 1 h after injection partially reduced mechanical hyperalgesia and allodynia, whereas giving it before intradermal capsaicin injection resulted in a clear reduction of both abnormal responses (Sun et al., 2003). Thus, it was concluded that CGRP is involved in both the development and maintenance of hyperalgesia.

Intradermal injection of capsaicin in rats was also used to demonstrate the importance of second-messenger cascades in central sensitization. Sluka and Willis (1997) found that capsaicin-evoked mechanical allodynia could be reversed by both G protein and protein kinase inhibitors. Kinase activity can result in post-translational modification of ion channels and receptors as well as increased trafficking and cell-surface expression. Further studies of specific kinase inhibitors after capsaicin injection has helped define precise mechanisms that may underlie central sensitization; for example, Zou et al., (2002, 2004) revealed that capsaicin-evoked PKA and PKC activation resulted in phosphorylation of the NMDA receptor subunit NR1 at Ser890/897 and Ser896, respectively, which could be blocked with specific inhibitors. A role for PKB/Akt was also found using the intradermal capsaicin model when Sun et al. (2007) showed elevated levels 5 min after injection in the DRG. This resulted in sensitization of WDR cells in the DH—leading to mechanical hypersensitivity—that could be prevented with a PKB inhibitor.

Fang et al. (2005) demonstrated a role for another kinase—CaMKII—in central sensitization. They showed that phosphorylation of cAMP response element-binding protein (CREB) 20 min after capsaicin injection could be blocked by the intrathecal CaMKII inhibitor 2-(N-[2-hydroxyethyl])-N-(4-methoxybenzenesulfonyl)amino-N-(4-chlorocinnamyl)-N-methylamine (KN-93). CaMKII may phosphorylate the glutamate receptor 1 subunit of AMPA receptor, resulting in increased surface expression and conductance of the channel. Up-regulation of phosphorylated CREB may also lead to enhanced gene expression and transcription of products that contribute to central sensitization.

Protein phosphatases are believed to oppose kinase action and can therefore control the duration of states such as central sensitization. Zhang et al. (2006) used inhibitors of protein phosphatase type 2A after capsaicin injection, which increased the duration of capsaicin-evoked hypersensitivity to mechanical stimuli by up to 3 h, thus suggesting that phosphatases indeed play a role in regulating central sensitization.

Another possible modulator of central sensitization that has been proposed is reactive oxygen species (ROS). The intradermal capsaicin model was used to examine their involvement in secondary hyperalgesia by administering systemic and intrathecal ROS scavengers after injection. Although systemic administration had no effect on either primary or secondary hyperalgesia, the intrathecal injection reduced activity in WDR cells and signs of secondary hyperalgesia (Lee et al., 2007). The study suggests that ROS may have a role in the induction or maintenance of central sensitization at the level of the spinal cord.

An additional mechanism believed to be involved in both peripheral and central sensitization, dorsal root reflexes (DRRs), have also been examined using intradermal capsaicin. Zou et al. (2001) showed that capsai-

cin-evoked NMDA receptor activation could in turn increase spinal GABA-ergic activity. It is believed that GABA release from spinal interneurons may induce DRRs through activation of receptors on the peripheral afferent fibers (PAFs) and thus depolarize the cells (Li et al., 2008). Li et al. (2008) further suggested that DRRs may be responsible for release of neuropeptides such as CGRP and thus contribute to the sensitization of PAFs. Removal of DRRs inhibited capsaicin-evoked sensitization, which was restored by administration of CGRP. Therefore, it can be concluded that DRRs are induced by central mechanisms and involved in sensitization of peripheral fibers.

Central sensitization is not only a phenomenon of ascending pain tracts but also is believed to be contributed to by the descending pathways. Serotonin (5HT) is released from descending neurons into the DH, and one action occurs via $5HT_7$ receptors to evoke an antinociceptive affect. Capsaicin-induced hypersensitivity was reversed in mice with the addition of a $5HT_7$ agonist and promoted when combined with a $5HT_7$ antagonist (Brenchat et al., 2009). This implies that 5HT is important in the development of mechanical hypersensitivity. Furthermore, administration of a $5HT_7$ receptor agonist in rats with capsaicin-induced sensitization showed a reduction in mechanical hypersensitivity when given systemically or intrathecally—however, intraplantar injection of the agonist increased hypersensitivity (Brenchat et al., 2012). This reduction suggests that during a state of central sensitization, although at the spinal level $5HT_7$ is antinociceptive, in the periphery, it is pronociceptive. Because oral administration of the agonist resulted in a decrease of mechanical hyperalgesia, it was concluded that the over-riding effects of $5HT_7$ activation are antinociceptive.

It is important to note that although 5HT is known to be both anti- and pronociceptive within the spinal cord (depending on the subtype of receptors activated), the predominant CNS role is thought to be in the facilitation of nociceptive signaling (Bannister et al., 2009). This action is believed to be largely modulated by $5HT_3$ receptors located mainly on afferent fibers terminating in the superficial laminae of the spinal cord, activated by 5HT released from descending pathways originating in the brain stem as well as on spinal inhibitory interneurons. $5HT_3$ is a ligand-gated ion channel, and activation results in an influx of Ca^{2+} to the peripheral fiber, which in turn may lead to increased release of neuropeptides such as SP, neurokinin A, and CGRP. In contrast, noradrenaline released from descending neurons is accepted to have a predominantly inhibitory role, via its
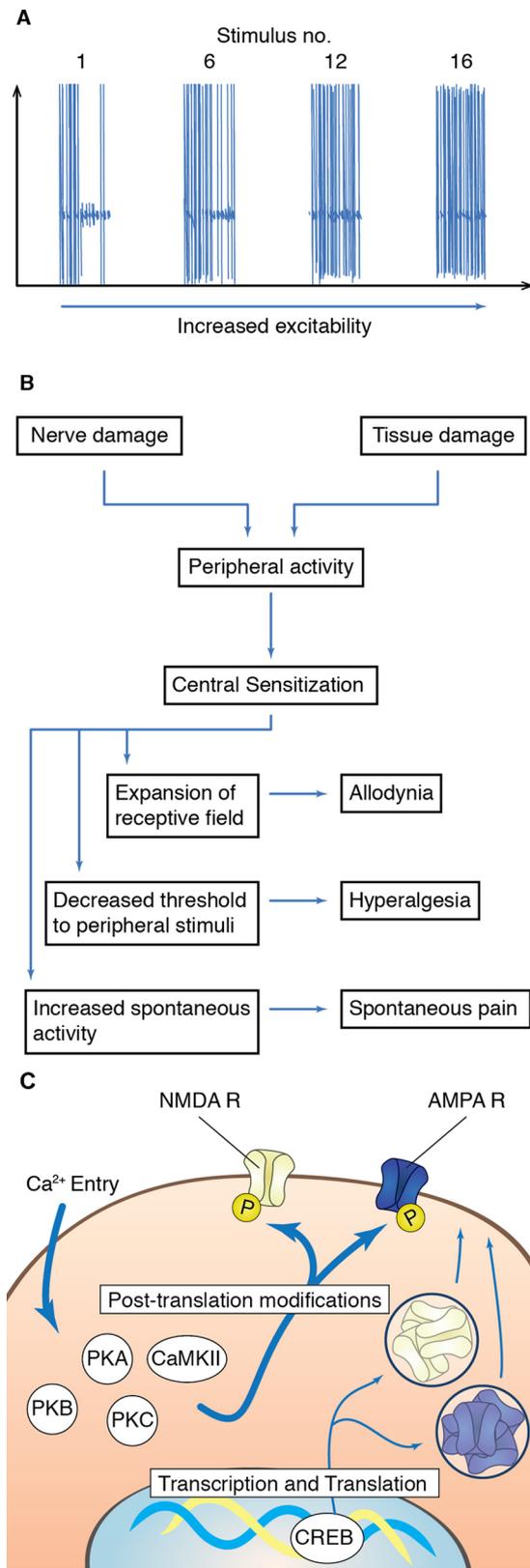
FIG. 9. Cellular events underpinning central sensitization. A, an example of wind up in a single neuron, which is believed to be a key event for the induction of central sensitization. B, the flow of neuronal changes and their behavioral correlates. C, intracellular mechanisms play a role in the development of central sensitization. Excess Ca^{2+} entry into projection neurons, mainly through the NMDA receptor, results in activation of Ca^{2+}-dependent enzymes such as PKA, PKB, PKC, and CamKII, which phosphorylate NMDA and AMPA receptors, both enhancing activity

directly by promoting channel opening as well as increasing trafficking and insertion into the postsynaptic membrane. Furthermore, activation of the transcription factor CREB may also be involved, which results in increased gene expression and thus production of receptors. (Other heterosynaptic mechanisms are outside the scope of this review.).
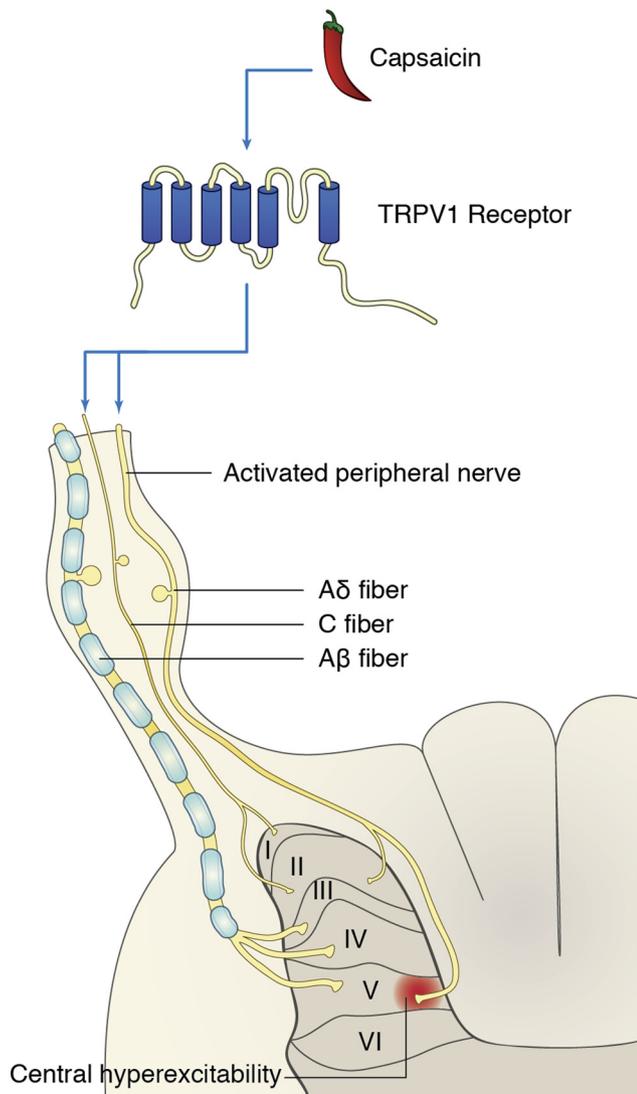
FIG. 10. Capsaicin-induced hyperexcitability of dorsal horn neurons. Peripheral application of capsaicin (such as by intradermal injection) excites afferent Aδ and C fibers. The ongoing input into the dorsal horn of the spinal cord is believed to underlie the development of hyperexcitability of spinal neurons and leads to secondary hyperalgesia and allodynia.

activation at α_2 adrenergic receptors in the DH. The balance between the two may be altered in chronic pain states and has been suggested to play a key role in supporting ongoing pain (Bannister et al., 2009). It is currently unclear how descending facilitation contributes to capsaicin-induced central sensitization, but it could be interesting to further investigate—particularly given that it is not essential for induction of LTP but has been shown to modulate its expression (Rygh et al., 2006).

The intradermal capsaicin model has yet to be used extensively to study supraspinal mechanisms, which are currently less well understood than the periphery in terms of pain processing. Moylan Governo et al. (2006) used fMRI to examine the brain response up to 120 min after injection. They found that initially the injection increased activity in the thalamus, PAG, parabrachial

nucleus, and superior colliculus. Mechanical stimuli applied after injection elicited a reduced blood-oxygen-level dependence signal in the PAG (Moylan Governo et al., 2006), which is unexpected because it is generally accepted that there is enhanced activity in the PAG in a state of central sensitization (Zambreanu et al., 2005). It was suggested this might simply be the oxygen demand outweighing the supply. Further studies of the effects of capsaicin-evoked central sensation on supraspinal sites could be of key interest.

The capsaicin model in animals clearly evokes central phenomena such as mechanical hypersensitivity, which are likely to be underpinned by the mechanisms involved in central sensitization detailed above. These are likely to be important with regard to chronic pain disorders, which involve changes in central processing of pain, including enhanced responses to peripheral stimuli. Of particular interest is the observation that it has been shown to evoke similar sensory changes in healthy human volunteers. Thus, this model is of great clinical relevance.

B. Humans

The use of capsaicin in experimental human pain models is imperative in the study of both processing and modulation of pain signals, and it has been used widely in both basic and clinical studies. It provides a unique reversible drive for central sensitization. Major advantages of experimental models are the possibility to control localization, intensity, frequency, and duration of stimuli, hereby providing quantitative measures of the psychophysical or neurophysiologic responses (Drewes et al., 2003a; Arendt-Nielsen et al., 2007; Brock et al., 2009). Such valid and reproducible experimental pain models, which mimic the clinical situation to a high degree, are valuable tools in investigating basic mechanisms as well as investigating effects and mechanisms of analgesics in humans (Arendt-Nielsen et al., 2007). The use of capsaicin has been developed to simulate aspects of clinical pain, because it leads to central sensitization and the associated symptoms—hyperalgesia and allodynia—that are both essential characteristics in chronic pain (Fig. 11. Capsaicin has mostly been administered topically, by intradermal or intramuscular injections, or orally in visceral models (LaMotte et al., 1991; Liu et al., 1998; Witting et al., 2000; Dirks et al., 2003; Brock et al., 2010).

C. Superficial Somatic Pain

Topical and intradermal administration of capsaicin produces a remarkable set of sensory alterations, including ongoing burning and aching pain, thermal and mechanical hypersensitivity, and allodynia in the surrounding skin (see Table 1 (Simone et al., 1989; LaMotte et al., 1991; Koltzenburg et al., 1992; Kilo et al., 1994). The models involve development of both primary and secondary hyperalgesia. Although primary hyperalgesia
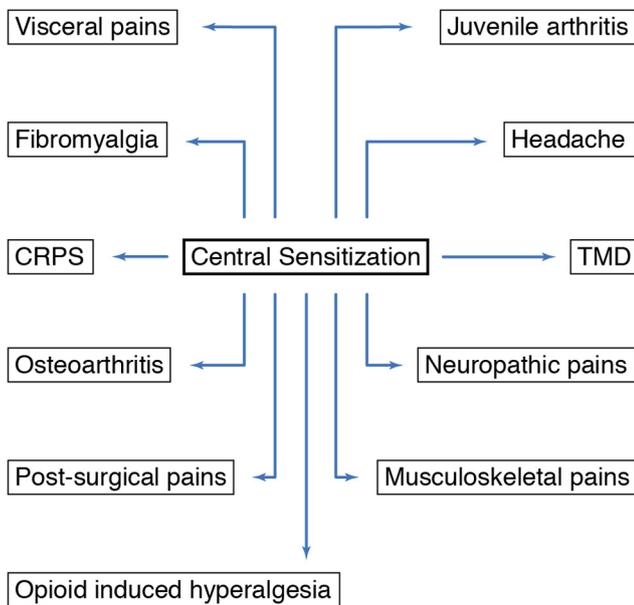
FIG. 11. Pathophysiological pain conditions in which central sensitization has been implicated. Central sensitization is believed to be a key phenomenon contributing to the development of a number of these chronic pain conditions. Increased knowledge of CS may therefore lead to the development of new compounds, which can help improve the outcome of such conditions.

is believed to be the net result of combined peripheral and central sensitization, secondary allodynia and hyperalgesia, by contrast, are believed to exclusively represent the phenomenon of central sensitization. It is believed this requires ongoing input from the primary area, to be maintained (Petersen and Rowbotham, 1999). The necessity of ongoing input to maintain sensitivity has been demonstrated in a human experimental hyperalgesic pain model in which rekindling with heat was crucial to sustain secondary hyperalgesia (Petersen and Rowbotham, 1999). This has further been confirmed in other studies demonstrating that reducing or terminating the ongoing input—such as by cooling or local anesthetics cause the area of hyperalgesia to shrink (LaMotte et al., 1991; Koltzenburg et al., 1992; Dahl et al., 1993).

As mentioned previously, it can be hypothesized that descending facilitation may play a role when the peripheral drive wears off after capsaicin-induced hyperalgesia; however, this is yet to be addressed in human models. It is tempting to consider equivalent the central sensitization underlying hyperalgesia and temporal summation (windup) (McMahon et al., 1993; Urban et al., 1994; Price, 1996); however, this has been questioned because different synapses are involved (Treede and Magerl, 1995). Magerl et al. (1998) suggest that the developed secondary hyperalgesia is a result of heterosynaptic facilitation as the enhanced responsiveness of spinal neurons mediated by low-threshold mechanoreceptors are not involved in the intense C-fiber input. This is independent of the mechanism involved in temporal summation, which affects only the same synaptic input that has transmitted the intense stimulus,

indicating homosynaptic facilitation. Intradermal capsaicin administration in humans has been investigated regarding reliability, reproducibility, and sensitization, and analysis of underlying mechanisms has been extensive (Hughes et al., 2002). Scanlon et al. (2006) investigated capsaicin-induced spontaneous pain corresponding to C-fiber activity, pin-prick hyperalgesia (quantified and evoked by use of e.g., von Frey filaments), which is mediated by C- and Aδ-fibers, and finally allodynia (evoked by gentle brush stimulation) primarily mediated by Aβ-fibers. In addition, electrophysiological studies have provided strong evidence against any sensitization of primary nociceptors in undamaged skin (Scanlon et al., 2006).

Intradermal capsaicin is often used as a tool to induce central sensitization and its physiological counterpart secondary hyperalgesia. However, spontaneous pain during intradermal capsaicin injection has been thought to influence the results. To address this issue, Jantsch et al. (2009) investigated the characteristics of memory for the sensory-discriminative components of the model. The study demonstrated reliable memory for magnitude and duration of intradermal capsaicin, supporting validity of the model. In addition, Lee et al. (2008) studied the blood-oxygen-level dependence fMRI response to intradermal capsaicin, in which all subjects experienced intense burning pain immediately after injection, and 80% (12/15) developed secondary mechanical punctate hyperalgesia, caused by central sensitization. The authors suggest that, similar to findings in animal models, brainstem activity is involved in maintenance of central sensitization, whereas cortical activity, primarily somatosensory cortex, reflects the increased intensity of pain experienced during capsaicin-induced central sensitization (Lee et al., 2008). To investigate secondary hyperalgesia, Torebjörk et al. (1992) applied an intradermal capsaicin injection combined with selectively stimulating intraneural low-threshold mechanoreceptors. The group showed a sensation of touch elicited in normal skin, whereas the same stimulus became painful when the receptive field was part of the secondary hyperalgesic area. In addition, intradermal capsaicin in humans was used to characterize the supraspinal contributions to central sensitization using functional magnetic resonance imaging technique. Zambreanu et al. (2005) found increased activity after intradermal injection in the contralateral brainstem, cerebellum, bilateral thalamus, contralateral primary and secondary cortices, bilateral posterior insula, cingulated cortex, right middle frontal gyrus, and right parietal association cortex. Brainstem activation was localized to two distinct areas of the midbrain reticular formation, nucleus cuneiformis and PAG, which are the major sources to communicate with RVM and therefore in an ideal position to modulate its output (Zambreanu et al., 2005).

Another role of intradermal capsaicin was studied by Gazerani et al. (2005), who applied capsaicin in the forehead to induce trigeminal sensitization as a model of

human migraine (Fig. 12). Capsaicin induced trigeminal sensitization and evoked gender-specific sensory and vasomotor responses; women generally showed the strongest manifestation during menstruation (Gazerani et al., 2005). It has been hypothesized that botulinum toxin type A (BoNT-A) inhibits peripheral sensitization of nociceptive fibers and indirectly reduces central sensitization. Hence, BoNT-A administration was incorporated in the above-mentioned migraine model, showing reduction of capsaicin-evoked pain, flare, skin temperature, blood flow, and area of secondary hyperalgesia, reflecting BoNT-A attenuation of neurogenic pain mechanisms (Gazerani et al., 2006).

Finally, the fact that a nonpainful stimuli (von Frey/brush) leads to painful sensation is a phenomenon believed to be produced as a consequence of central sensitization, and it is present in many clinical pain states (e.g., postherpetic neuralgia, complex regional syndrome). A model of intradermal capsaicin-induced allodynia is therefore highly useful in clinical studies investigating analgesic effects. However, it is crucial that the dose of capsaicin produces consistent neurosensory measures. In addition, use of 100 μg has shown to be reproducible and valid over time, important factors in clinical studies investigating mechanisms and effects of analgesics and antihyperalgesics (Simone et al., 1989; Hughes et al., 2002).

Because of the ability of intradermal capsaicin to produce symptoms attributed to a number of different chronic pain conditions, it has also been widely used in studies testing analgesic effect of pharmacological compounds. However, it is well recognized that healthy volunteer responses are greatly variable in their development of central sensitization—these differences may be due to differences in enzymes required for the breakdown of capsaicin or in the TRPV1 receptors themselves, among a number of other possible mechanisms. Therefore, to optimize a pharmacological study design, Klein et al. (2008) used enriched enrolment, in which they included only 78% of those subjects who did develop hyperalgesia. The remaining 22% did not develop the required hyperalgesia, which was defined as at least a 2-fold increase in pain rating to pin-prick stimuli after a 40-μg intradermal capsaicin injection (Klein et al., 2008).

Andresen et al. (2011) demonstrated that red-haired women were less sensitized to capsaicin-induced hyperalgesia compared with blonde/dark haired women. Because this phenotype is known to come from mutations in the MC1 receptor, which is expressed in the brain and is believed to be involved in the response to endorphins, it suggests that some central opioid mechanisms may be involved in the modulation of the capsaicin response and therefore may be of importance in future studies investigating antihyperalgesic drugs as well as treatment of pain (Andresen et al., 2011).

Topical and intradermal injection of capsaicin have been shown to be valid models for testing antihyperalgesic effect of analgesics (Scanlon et al., 2006; Staahl et al., 2009a,b). Even though Magerl et al. (1998) demonstrated that hyperalgesia and wind-up are independent regarding synaptic facilitation, numerous human experimental pain studies with ketamine—an NMDA receptor antagonist—have shown effects of experimentally induced hyperalgesia as well as temporal summation (wind-up) (Staahl et al., 2009b). It may be speculated that temporal summation is partly needed to develop hyperalgesia; therefore, it is not surprising that repetitive electrical stimulation (temporal summation) can lead to hyperalgesia.

An important imaging study with fMRI in normal volunteers studied effects of gabapentin, effective in neuropathic pain, on brain processing of pain signals in normal and central sensitization states, the latter being induced by capsaicin. The effect of gabapentin was to modulate activation in cortex, independently of the presence of central sensitization, whereas the drug reduced brainstem activity but only during central sensitization (Iannetti et al., 2005). This suggests changes in descending controls as discussed previously (Bannister et al., 2009). Capsaicin also produced deactivation, a mechanism wherein the baseline brain activity is reduced, potentially highlighting the pain signals—this was suppressed by gabapentin only during central sensitization. This effect was more robust than the effect on brain activation. Thus, in healthy volunteers, central sensitization has brain correlates and gabapentin can modulate this activity.

It is important to keep in mind that intradermal injection of capsaicin is—as mentioned above—associated with extreme pain, and the topical heat/capsaicin model has been developed to achieve a noninvasive paradigm to generate stable, long-lasting, and reproducible primary and secondary hyperalgesia. This model is furthermore suitable for testing of analgesics with longer absorption than intravenous administration (Dirks et al., 2003).

D. Experimental Deep Somatic Pain

This section will focus on capsaicin evoked muscle-related pain. Clinically, muscle pain may cause local (e.g., injury, myofascial pain) or widespread (whiplash injury, fibromyalgia) pain, which can be presented with or without referred pain (Arendt-Nielsen and Yarnitsky, 2009); consequently, experimental models are needed to mimic the clinical setting.

As stated, capsaicin excites the nociceptive afferents and causes neurogenic inflammation, primary heat and mechanical hyperalgesia, and secondary mechanical hyperalgesia (Baumann et al., 1991; LaMotte et al., 1991; Gazerani et al., 2005). Short-lasting (minutes) muscle hyperalgesia can be studied after intramuscular injections of capsaicin (Arima et al., 2000; Sohn et al., 2000;

TABLE 1
Human studies using intradermal capsaicin

Amount/Test Devices	Method	Results	Reference
250 µg Artist's Number 2 brush (1 cm/s from a point 10 cm away); Von Frey hair (microfilament bending threshold 196 mN at a point 10 cm away)	Capsaicin was injected intradermally in the left and right arm of 12 healthy male volunteers. Spontaneous pain response and areas of allodynia and hyperalgesia were measured.	Little variability in pain perception and allodynia was observed. With each subsequent injection, variability was observed for pinprick hyperalgesia The nondominant arm might be more sensitive to pain than the dominant arm.	Hughes et al. (2002)
0.05, 1, and 20 µg	Injection was performed across three sessions 1 week apart. Pain ratings were recorded immediately or 1 h or 1 day after injection. Subjects recalled their pain 1 week later.	The strong burning pain of the injection declined within a few minutes. Subjects were able to reliably discriminate pain magnitude and duration. This was also the case for pain recall.	Jantsch et al. (2009)
10 µg Nylon filament (225 mN)	Microelectrodes were inserted in the peroneal nerve just below the knee, measuring the nerve activity after capsaicin injection. Pain intensity to each stimulus was assessed. Spontaneous pain to injection of capsaicin was recorded for the first 3 min. Pinprick hyperalgesia was measured.	Intradermal application close to or inside the receptive field led to higher discharge rates. Discharge rates decreased by mechanical stimulation. Pain was positively correlated to high discharge rates. Activity in C mechano-heat nociceptors contributed to the magnitude and duration of pain evoked by intradermal injection of capsaicin. After-effects were concentration related: high concentration led to desensitization, low concentration led to sensitization.	LaMotte et al. (1992)
250 µg	Capsaicin was injected in the volar forearm and dorsal foot. Temperature was fixed or varied followed by observation of pinprick hyperalgesia and brush stroke. Skin blood flow was measured.	Stabilized skin temperature before injection decreased variability. A lower concentration of capsaicin injected in the forearm led to a decreased between-session consistency. The dorsal foot was more sensitive than the volar forearm. Blood flow in the dorsal foot increased with reclining position.	Liu et al. (1998)
10–25 of 10 mg/ml Semmes-Weinstein monofilament (26g)	Capsaicin was injected in the forearm or thigh. Spontaneous pain intensity was recorded at time of injection and every 5 min up to 20 min. Area of hyperalgesia was assessed.	Spontaneous pain intensity declined over time. A persistent area of secondary tactile allodynia was observed.	Modir and Wallace (2010)
50 µg Von Frey filament (60g)	14 Healthy volunteers (9 men, 5 women) were tested for in regions of the forearm: cold stimuli (30, 20, 10, and 0°C) by a squared contact thermode before and after capsaicin injection. Hyperalgesia after an intradermal capsaicin injection by testing with punctuate stimuli (von Frey filament). Skin blood flow (laser Doppler flowmetry) and temperature (infrared camera) were measured before and after capsaicin injection.	Increase in blood flow after baseline cold stimulation at the 0°C compared with the three other sides. In addition, vasodilatory effect was found after the capsaicin injection compared with baseline for all regions.	Pud et al. (2005)
0.1, 1, 10, or 100 µg	Pain scores were recorded 0, 5, 10, 15, and 60 min after injection. Areas of mechanical allodynia and pinprick hyperalgesia were quantified 15 and 60 min after injection.	Capsaicin produced dose-dependent increases in spontaneous pain, the area of mechanical allodynia, the area of pinprick hyperalgesia, and visual flare. 10 µg produced significantly more pain than 1 µg. 100 µg produced more pain than 10 µg, but not significantly.	Scanlon et al. (2006)
0.1–100 µg	Capsaicin injection into the forearm. Pain threshold to heat and hyperalgesia was assessed.	Heat pain threshold was lowered in the injection site. Magnitude and duration of hyperalgesia was dose dependent.	Simone et al. (1987)

TABLE 1—*Continued.*

Amount/Test Devices	Method	Results	Reference
250 μg	Capsaicin-evoked pain intensity was assessed every 1 min for 5 min and every 5 min the following 30 min. 5, 30 and 60 min after injection, temperature and blood flow were measured, followed by assessment of visual flare and hyperalgesia. Secondary hyperalgesia, stroking (cotton swab) and heat was assessed.	Blood flow and temperature increased after injection. Areas of visual flare, stroking hyperalgesia, and punctuate hyperalgesia were covered by the area of significantly increased bloodflow detected 5 min after injection. There was no difference in pain intensity to radiant heat inside/outside the area of punctuate hyperalgesia	Sumikura et al. (2003)
10 μg	Spontaneous pain and secondary mechanical hyperalgesia was assessed after injection. Melittin injection (a main compound of bee venom) was compared to injection of capsaicin.	Capsaicin and melittin evoked comparable spontaneous pain. Larger areas of mechanical hyperalgesia was observed after injection of capsaicin compared with melittin.	Sumikura et al. (2003)
10 μg 0.5–1.5 cm soft hair brushes	Capsaicin was injected in the volar part of the nondominant arm. Continuously perceived pain was assessed. Heterotopic stimulation with cold water that was nonpainful or painful (induction of diffuse noxious inhibitory control) was applied to the foot. Experiment 1, continuous brush-evoked pain intensity was scored together with capsaicin injection Experiment 2, continuous brush-evoked pain intensity was scored alone.	Immersion of the foot into cold water reduced capsaicin-evoked pain intensity and brush evoked pain but not the area of brush-evoked pain.	Witting et al. (1998)
10 μg Brush (0.7 cm/s and 0.15–0.20 N/cm²) and von Frey size (75, 86 g)	Capsaicin was injected intradermally in the volar part of the forearm in 17 healthy volunteers. Injections: 7–8 cm proximal from the wrist at four sites as a square. Experiment 1, 6-, 15-, or 24- min intervals Experiment 2, distance of 0.5 or 4 cm Pain intensity and areas of allodynia and hyperalgesia were measured.	The response to intradermal injection of capsaicin was dependent on time and distance between injections. Lack of significant relation between capsaicin pain intensity, area of allodynia. and hyperalgesia suggest different central mechanism.	Witting et al. (2000)
40 μg 200-μm diameter probes (35–407 nM)	Blockade of the superficial radial nerve, followed by capsaicin injection. Secondary hyperalgesia was detected by pinprick.	Pricking pain by punctuate stimuli was reduced by 75%, when A-fiber were fully blocked but with fully intact C-fiber. Burning pain to capsaicin injection remained unchanged. Hyperalgesia to punctuate stimuli was detectable immediately after block release, when A-fiber conduction returned to normal.	Ziegler et al. (1999)

Witting et al., 2000; Graven-Nielsen and Mense, 2001). Psychophysical assessments of muscle hyperalgesia can be done by drawings, which describes localization and extent of pain areas, pain intensity assessed on a visual analog scale or quantitatively with pressure algometry (Arendt-Nielsen and Yarnitsky, 2009). However, more
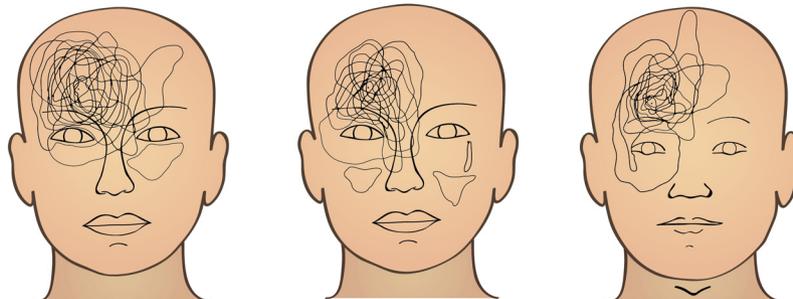

Fig. 12. Capsaicin was injected intradermally in the forehead to induce trigeminal sensitization as a model of migraine. Capsaicin induced sex-specific sensory responses, here assessed as referred pain areas, which was most apparent in women during menstruation (left, menstrual phase; center, luteal phase; right, men).

objective methods such as measuring blood flow, recording electromyography, and electroencephalography (EEG), also exist (Chang et al., 2004; Arima et al., 2009). A model of deep tonic pain originating from the masseter muscle, mimicking clinical pain (such as, for example, in temporomandibular joint disease), was established by injection of 0.2 ml of a 100 μg/ml capsaicin preparation into the deep mid-portion of the muscle (Arendt-Nielsen and Yarnitsky, 2009). The capsaicin-induced pain was described as "sharp", "pulsing," and "shooting."

After capsaicin administration, subjects tolerated significantly lower pressure stimulation of the masseter, which demonstrates sensitization to mechanical stimuli after the intramuscular injection (Arendt-Nielsen et al., 2008a). The finding is supported by other studies (LaMotte et al., 1991; Witting et al., 2000; Sluka, 2002; Arima et al., 2009). Central integration has also been shown: if intramuscular chemical stimulations are repeated, pain intensity and the referred pain area are increased, indicating temporal summation (Graven-Nielsen et al., 1997).

Arima et al. (2009) investigated the effect of capsaicin-evoked masseter-muscle pain on intramuscular blood flow at rest and during contractions. Blood flow was investigated by a single-fiber laser Doppler probe inserted to the masseter muscle and on the skin before and after administration of intramuscular capsaicin and performance of isometric voluntary muscle contractions (Arima et al., 2009). Intramuscular blood flow increased immediately after capsaicin injection but decreased rapidly (30 s) to preinjection values. Cutaneous blood flow above injection site was increased for 10 min, whereas peripheral blood flow (finger) was decreased. Muscle contractions were associated with increases in intramuscular blood flow before and after the capsaicin injection (Arima et al., 2009). The study demonstrated that increased blood flow by muscle and neurogenic inflammation in muscles could possibly be mediated via antidromical effects and local release of vasoactive substances (Arima et al., 2009). It is well known that the muscular glutamate receptor is involved in muscle pain, because glutamate-evoked pain can be reduced by coadministration of an NMDA antagonist (Cairns et al., 2006; Castrillon et al., 2008a,b). Hence, to explore the interaction between glutamate and capsaicin, a glutamate injection before or after the capsaicin administration was incorporated in the model (Arendt-Nielsen et al., 2008b). The findings showed that glutamate injection after capsaicin evoked significantly less pain compared with its injection after isotonic saline. In contrast, the capsaicin injection after glutamate evoked significantly stronger pain compared with its injection after isotonic saline (Arendt-Nielsen and Yarnitsky, 2009). Hence, the authors suggest a strong interaction between the peripheral glutamate excitatory receptors and TRPV1, which possibly could influence the magnitude of development of clinical musculoskeletal pain (Arendt-

Nielsen and Yarnitsky, 2009). In another study by Wang et al. (2010), the interaction between glutamate and capsaicin-evoked muscle pain on human jaw motor functions was assessed. Objective assessments such as resting electromyography, maximum voluntary bite force, and maximum voluntary jaw opening were recorded before and after administration of glutamate or capsaicin or isotonic saline, in a paired-sequence order. Psychophysical pain intensity before injection was recorded on a 0 to 10 numerical rating scale during each maximum voluntary bite force and jaw opening and subsequently at 5-min intervals for 50 min (Wang et al., 2010). Glutamate followed by capsaicin injection evoked increased resting electromyography activity and higher peak pain. Saline/glutamate followed by capsaicin evoked decreased bite force and bite opening. The results indicate that intramuscular administration of glutamate and capsaicin induces muscle pain, which has the potential to perturb some normal jaw motor functions (Wang et al., 2010).

Finally nociceptive input to the brain deriving from intradermal or intramuscular injection of capsaicin to the forearm has been investigated. Visual Analog Scale score, EEG topography, and power spectra were acquired before and during the vehicle/capsaicin injection (Chang et al., 2004). Visual Analog Scale profiles for skin and muscle pain were highly similar despite distinct qualities perceived, and both applications produced a similar but not identical EEG topographic pattern. However, muscle pain induced increased β-2 activity in the extensive frontal, parietal, and occipital areas compared with skin pain, and the authors suggest that the nociceptive inputs from muscle and skin are processed differently in the similar neural matrix of the brain (Chang et al., 2004).

E. Human Visceral Studies

An overview of studies in which capsaicin was used to investigate human experimentally induced visceral hyperalgesia is given in Table 2. Results obtained from human tissues strongly differ from those obtained from animal preparations (Barthó et al., 2004). For example, Hammer et al., (1998) stated that the ability of intrajejunal capsaicin to give rise to pain was unexpected, because exposure of the rat gastric mucosa to 5-fold higher concentrations of capsaicin failed to evoke pseudoaffective reactions indicative of pain. Unfortunately, results obtained on human tissues are sparse, and the capsaicin mystery is therefore somehow still unsolved regarding specific effects in humans. However, interesting knowledge on general effects has been gained from human studies.

Lee et al. (2004) found no effect of capsaicin on the first perception of intragastric distension, but capsaicin infusion decreased the pressures and corresponding wall tensions at the threshold of discomfort but, in contrast to the findings of Gonzalez et al. (1998), with-

TABLE 2
Capsaicin induced visceral hyperalgesia

Capsaicin Formulation	Method	Results	Reference
100 μg of capsaicin was dissolved in 1 ml of liquid and was given in volumes of 1, 2, 3… 10 ml.	Capsaicin was applied topically to the mucosa 5 cm in the gut by a plastic catheter via the stomal opening in patients with ileo- or colostomy.	Capsaicin evoked pain and referred pain areas in all subjects. Moreover, sympathetic manifestations were quantified by significant increases in the blood flow and temperature of the referred area.	Arendt-Nielsen et al. (2008)
180 ml acid (HCL, 0.1 M) and 2 mg capsaicin in 20 ml of solvent (10 ml/min).	Distal esophageal chemical perfusion (7 cm above lower esophageal sphincter).	Rectal hyperalgesia to heat and mechanical stimulations were demonstrated after chemical perfusion of the esophagus.	Brock et al. (2010)
Increasing volumes of capsaicin 50 μg/ml (0.25–3 ml)	Capsaicin was applied to the ileum via the stomal opening.	Hyperalgesia was found to cause distension of the gut after capsaicin application.	Drewes et al. (2003)
2.5 ml Tabasco capsaicin-containing red pepper sauce suspension.	Intraesophageal application of a capsaicin solution (10 cm above the lower esophageal sphincter).	Decreased perception and discomfort threshold of intraesophageal balloon distension.	Gonzalez et al. (1998)
180 ml of acid (HCL, 0.1 M) and 2 mg of capsaicin in 20 ml of solvent (10 ml/min).	Intraesophageal perfusion (7 cm above lower esophageal sphincter).	Results from evoked brain potentials following painful electrical stimulation of the rectosigmoid colon demonstrated visceral hypersensitivity as increased EEG power in the delta, theta, and alpha frequency bands were found.	Graversen et al. (2011)
Capsaicin (200 μg/ml, 2.5 ml/min). Duration of perfusion was 60 min or until discomfort.	Intraluminal capsaicin in different regions of the upper gastrointestinal tract.	In the duodenum, barostat volumes at sensation and discomfort were comparable before and after capsaicin perfusion, whereas in the jejunum balloon volumes were lower after capsaicin infusion.	Hammer and Vogelsang (2007)
Pretreatment: capsules (0.5 mg capsaicin) three times per day for 7 days. Capsaicin perfusion: 40 μg/ml (infusion rate of 2.5 ml/min). Duration of perfusion was 60 min or until discomfort.	Before and after capsule ingestion, the jejunum was distended with a balloon and perfused with a capsaicin solution.	Distension with 40 ml induced lower perception scores after 1 week of capsaicin treatment (desensitization). During capsaicin perfusion, discomfort thresholds were reported earlier after 1 week of capsaicin treatment (sensitization).	Hammer (2006)
3 ml of a 0.17 mg/ml capsaicin solution, diluted with saline to a total volume of 10 ml	Intraesophageal capsaicin installation	No differences in symptom pattern or intensities induced by esophageal acid perfusion were found and neither was sensitivity to esophageal balloon distention.	Kindt et al. (2009)
0.84 mg capsaicin in 5 ml Tabasco capsaicin-containing red pepper sauce (10 ml/min.)	Capsaicin was applied in the gastric fundus.	After capsaicin perfusion, higher perception scores were reached for the same distending pressures by gastric barostat.	Lee et al. (2004)
180 ml of acid (HCL, 0.1 M) and 2 mg capsaicin in 20 ml of solvent (10 ml/min).	Distal esophageal chemical perfusion (7 cm above lower esophageal sphincter).	Reduction of the pain threshold to esophageal heat and electrical stimuli. Increase of the referred pain areas to esophageal mechanical and electrical stimulation.	Olesen et al. (2009)
Capsaicin solutions (40, 200, and 400 μg /ml) 2.5 ml/min were perfused for 60 min or until severe discomfort occurred.	Perfusion site at the ligament of Treitz and, 7 cm distally, a barostat balloon.	Repeated capsaicin (200 μg/ml) applications reduced time until discomfort occurred. Pain thresholds during distensions were not different before and after capsaicin perfusion.	Schmidt et al. (2004)

out a significant change in corresponding volumes. It cannot be excluded that different outcomes can be related to different methods for stimulation and assessment. Different modalities were used in the different studies: heat, electrical, chemical, and mechanical stimulation. For mechanical stimulation, several parameters can be assessed (e.g., balloon pressures, balloon volumes, and wall tension), and some studies looked into time until discomfort or pain occurred. However, despite different modalities and assessment methods, most human studies found a sensitizing effect of capsaicin, and only one study of 11 could not demonstrate sensitization to any modality (Kindt et al., 2009).

Intraesophageal application of capsaicin-containing red pepper sauce (Tabasco) has been found to decrease the distension volume for perception and discomfort thresholds of intraesophageal balloon distension. Nevertheless, the sensitizing effect of capsaicin disappeared 30 min after infusion. This return to basal level could be caused by limited local activity or by desensitization (Gonzalez et al., 1998). Desensitization was also found by Hammer (2006) in a timing study in which distension with 40 ml capsaicin induced lower perception scores after 1 week of treatment. However, the same study found that during capsaicin perfusion, discomfort thresholds were reported earlier after 1 week of capsaicin treatment, indicating sensitization. This could indicate that mechanoreceptors and chemoreceptors are affected differently by 7 days of oral ingestion of capsaicin capsules (Hammer, 2006).

Later, a purified solution of capsaicin has been used in different doses at different sites of the gastrointestinal tract. It has been concluded that capsaicin evokes abdominal sensations by stimulation of chemoreceptors and not mechanoreceptors (Schmidt et al., 2004). However, several studies have found reduction in pain thresholds to mechanical, electrical, and thermal stimulation (Gonzalez et al., 1998; Drewes et al., 2003b; Lee et al., 2004; Hammer and Vogelsang, 2007; Olesen et al., 2009; Brock et al., 2010). This indicates that capsaicin must affect a variety of receptors, and sensitization/desensitization is not necessarily dependent on the quality of receptor activation but more the quantity or duration. Nevertheless, this is still not clear from human studies, and further investigation may be useful. In this context, it should be noted that visceral innervation is weak and diffuse, and so the ability of capsaicin to activate sufficient numbers of TRPV1 afferents to induce changes may be more difficult in visceral tissue compared with somatic and muscular tissues (Sikandar and Dickenson, 2012).

Administration of capsaicin by itself or in a combination with acid has been shown to induce visceral hyperalgesia, which has led to hypersensitivity to heat and mechanical, electrical, and chemical stimulation (Gonzalez et al., 1998; Drewes et al., 2003a; Lee et al., 2004; Hammer, 2006; Hammer and Vogelsang, 2007; Olesen et al., 2009; Brock et al., 2010). These stimuli are likely to reflect both peripheral and central sensitization. Therefore, quantification of referred pain areas representing the viscerosomatic convergence to esophageal acid and capsaicin perfusion has been studied, and indications for central effects have been found (Olesen et al., 2010). These models of central sensitization were further developed to investigate viscerovisceral hyperalgesia, which manifests as extra segmental hypersensitivity to mechanical and heat stimulation of the rectum after esophageal infusion (Brock et al., 2010). Graversen et al. (2011) have, for the first time in humans, directly demonstrated central neuronal changes as a result of

chemical irritation by intraesophageal acid and capsaicin perfusion. This was demonstrated as an increased EEG power in the delta, theta, and alpha frequency bands. The authors found a correlation when taking all frequency bands into consideration simultaneously, which showed that central sensitization was directly reflected by the alteration of the CNS response after noxious stimulations, whereas no correlation to changes in pain score was seen for the placebo response. It is noteworthy that the correlation was evident even though some subjects developed hyposensitivity, where they tolerated higher stimulus intensity after perfusion with acid and capsaicin than before. This phenomenon of mixed hypo- and hypersensitivity to the same treatment has been demonstrated previously (Hammer, 2006) and can be explained by the two competing mechanisms involved with capsaicin administration—sensitization or desensitization, which will be discussed in the next section. It should be noted, however, that a combination of acid and capsaicin was applied. Therefore, it cannot be concluded whether the central effect was caused by capsaicin, acid, or the combinations. Further human studies are needed to distinguish between capsaicin and acid effects.

It has been discussed whether capsaicin causes pain by directly stimulating nociceptive afferents in the intestinal mucosa or by stimulating motility as might be deduced from the cramping nature of the sensation. Stimulation of motility may result in pain from excessive contractions or movement of the inflated balloon along the intestine. Excessive contraction or distension of the gut is well established to give rise to pain (Hammer et al., 1998). A first-in-human study has demonstrated that afferent nerves from human gut tissue ex vivo are stimulated directly by capsaicin as highly increased firing rates (Peiris et al., 2011). Hence, capsaicin can cause pain as a combination of direct stimulation of nociceptive afferents and stimulation of motility.

It is noteworthy that there appears to be a dramatic interaction with the sympathetic response in referred pain areas, which has been studied by application of capsaicin to human ileo- or colostomy (Arendt-Nielsen et al., 2008a). Capsaicin-evoked pain and referred pain areas in all subjects and sympathetic manifestations were quantified by increased temperature and blood flow of the referred area (Arendt-Nielsen et al., 2008a). Bowel disorders are also accompanied by sympathetic manifestations. Schmidt et al. (2004) demonstrated that their model of intraluminal capsaicin can modulate perception and cause discomfort or pain in a normal-appearing gut and thus provides a model for the study of functional bowel disorders.

Few human studies in which visceral sensitization was used as models for the study of disease have been used to study analgesic effects or mechanisms. Olesen et al. (2010) investigated the effects of morphine and oxycodone in ex-

perimentally evoked hyperalgesia in healthy volunteers. Hyperalgesia was induced by esophageal perfusion with acid and capsaicin. They found that morphine and oxycodone exerted different effects in the sensitized pain system. The evoked hyperalgesia bridged findings from studies in healthy volunteers to patients, and new fundamental knowledge on different analgesic effects in hyperalgesia was found.

Another approach can be to use these human pain models of sensitization as specific tools to investigate analgesic mechanisms. For example, Krarup et al. (2011) investigated the efficacy of a new transient receptor potential vanilloid 1 antagonist AZD1386 in a model of human esophageal pain where sensitization and activation of TRPV1 receptors was evoked by acid. The results demonstrated a dose-dependent increase in the esophageal heat pain threshold after administration of the TRPV1 antagonist (Krarup et al., 2011).

Willert et al. have used a model of human visceral pain hypersensitivity to determine whether the development and maintenance of human visceral hypersensitivity is NMDA receptor mediated. This was performed using the NMDA receptor antagonist ketamine either before or after acid esophageal infusion of acid. Acid-induced esophageal hypersensitivity was prevented by ketamine and ketamine administered after acid reversed the induction of esophageal hypersensitivity induced by acid (Willert et al., 2004). As capsaicin induces similar hypersensitivity and central sensitization, it might also be used in human pain models as a model of functional gastrointestinal disorders to confirm effects of NMDA antagonists or other putative analgesics.

XIII. Sensitizing or Desensitizing?

Activation of the TRPV1 receptor, in rats and humans, can cause both sensitization—as previously discussed—but may also result in desensitization of nerve cells. In 1977, Szolcsányi showed that capsaicin application could result in a long-lasting insensitivity to mechanical pain, which was believed to be due to peripheral mechanisms. In addition, when capsaicin is systemically administered in supratherapeutic doses to neonatal rodents, capsaicin has been found not only to desensitize sensory neurons but also to induce degeneration of the DRG sensory afferents (Jancsó et al., 1977). It has later been revealed that desensitization is a dose-dependent phenomenon, whereby repeated application of low dose or a single high dose of injected or topical capsaicin led to immediate desensitization, whereas single lower doses cause activation (LaMotte et al., 1991; Simone et al., 1998; Kennedy et al., 2010). It is also well characterized that although a single application of low-dose capsaicin cream (~1%) results in an activation of PAFs and both primary and secondary hyperalgesia, repeated application of topical capsaicin over a few weeks dampens re-

sponses to both heat and mechanical stimuli (Nolano et al., 1999; Mason et al., 2004). This desensitization seems selective for A and C fibers and hence supports the use of capsaicin as a tool to study nociceptive afferents and consequences of their deactivation.

There are a number of ways in which capsaicin can result in desensitization of TRPV1-positive afferents. Activation may cause an acute rapid desensitization, long-term tachyphylaxis, and could eventually be associated with a withdrawal of epidermal nerve fibers (ENFs) (Backonja et al., 2008). Both types of desensitizations occur in the area localized to the site of injection/application; however, the time frame for each varies; the initial acute desensitization occurs within the first 20 s after agonist binding, whereas tachyphylaxis and ENF withdrawal occur over much longer periods up to 72 h after application (Simone et al., 1998; Touska et al., 2011). The type of desensitization will depend on the concentration of capsaicin, length of application time, and the penetration through to the dermis (Touska et al., 2011).

Rapid desensitization first involves capsaicin binding of TRPV1, which not only activates PAFs and pain pathways but also results in release of neuropeptides from the C fiber terminals in the periphery. This includes CGRP and SP, which are involved in local neurogenic inflammation and thus an area of flare around the application site. Once released, terminals are depleted and the nerve may become desensitized (Maggi and Meli, 1988; Simone et al., 1998).

Second, voltage-gated ion channels are inactivated, which also results in a rapid, short-lasting, desensitization as further action potentials cannot be generated (Simone et al., 1998; Tominaga and Tominaga, 2005). It is believed that the desensitization of fibers is a protective mechanism to inhibit excessive calcium influx, which leads to excitotoxic cell death. As already discussed, activation of TRPV1 and entry of Ca^{2+} ions to the nerves activates a number of enzymes, which may lead to sensitization. However, a second effect is the activation of other Ca^{2+}-dependent enzymes such as protein phosphatase 2B/calcineurin, which is likely to lead to tachyphyaxis (Docherty et al., 1996; Mohapatra and Nau, 2005). Calcineurin dephosphorylates TRPV1 with a key site believed to be Thr370 and therefore decreases sensitivity to agonists such as capsaicin (Mohapatra and Nau, 2005).

The importance of Ca^{2+} in capsaicin-induced desensitization was highlighted by Docherty et al. (1996), who found that desensitization in cultured rat DRG neurons was strongly inhibited by the calcium chelator 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA). The study also showed that inhibition of calcineurin by cyclosporin A complexed to cyclophilin greatly reduced desensitization (Docherty et al., 1996). Koplas et al. (1997) demonstrated that reducing the external Ca^{2+} concentration resulted in lower levels of acute desensi-

tization and tachyphylaxis. However, caffeine-induced release of intracellular Ca^{2+} resulted in desensitization, even in the absence of extracellular Ca^{2+} (Koplas et al., 1997), therefore confirming that desensitization is dependent on both intra- and extracellular Ca^{2+} and a subsequent dephosphorylation of TRPV1. Desensitization can therefore involve a number of mechanisms and, as such, underlies some of therapeutic use of low-dose capsaicin cream.

A. Altered Skin Innervation after Transient Receptor Potential Vanilloid 1 Activation

Other than receptor desensitization, another mechanism can be brought into play by which the peripheral sensory innervation is altered by high levels of activation of the TRPV1 receptor. Simone et al. (1998) showed that intradermal injection of capsaicin into the skin overlying the shoulder results in a decrease of ENFs within a radius of 1 to 2 mm away from the injection site, up to 72 h after injection, therefore suggesting that withdrawal of ENFs from the dermis is restricted to fibers that have come into direct contact with capsaicin. However, based on assessment of ENF density in skin biopsies after topical exposure to an occlusive 35 × 50-mm bandage containing 1.8 g of 0.1% capsaicin cream, Polydefkis et al. (2004) found that capsaicin application produced denervation of both the epidermis and the subepidermal plexus, with few or no ENFs remaining after 48 h in both healthy control subjects and patients with diabetes. This withdrawal may be due to the previously described rearrangement of microtubules or simply to mitochondrial dysfunction. The rate of nerve regeneration was associated with the baseline ENF density, but not age, sex, epidermal thickness, or postcapsaicin treatment density. ENF regeneration, as determined by recovery of ENF density, occurred at a rate of 0.18 ± 0.08 fibers/mm per day in healthy control subjects, which was significantly reduced in subjects with diabetes (0.07 ± 0.06 fibers/mm per day, $P < 0.001$) after adjusting for changes in baseline ENF density (Polydefkis et al., 2004).

A loss of ENFs would result not only in a decreased peripheral activity through selective drawing back of pain fibers but might also decrease central sensitization driven by these fibers; therefore, the rationale of applying topical capsaicin to achieve analgesia may extend beyond receptor events. The clinical application of these approaches is discussed in section XIV.

XIV. Capsaicin As a Therapeutic Pharmacological Agent

Capsaicin has been used all over the world for many years as a pharmacological intervention for a plethora of ailments. It is used to treat fevers, nausea, vomiting, malaria, and different pain conditions. In regions of central Peru, the leaves are burned or used to create steam in the bath to cure headaches; in the Dominican Republic, the leaves are ingested to treat painful menses; and in the Phillipines, the fruit is used to treat arthritis (Meghvansi et al., 2010). This final section considers the use of capsaicin as a therapeutic option, but first discusses the use of TRPV1 modulation to elucidate the role of different fibers in the behavioral changes that accompany models of nerve injury. Two studies have employed the desensitization of TRPV1-positive fibers with systemic RTX to tease out the roles of TRPV1-positive and -negative fibers in animal models. Although many models of different pain conditions monitor and manipulate responses to evoked stimuli, many patients with neuropathic pain report stimulus-independent spontaneous pain, often described as burning. King et al., (2011) used RTX to prevent nerve injury-induced thermal hypersensitivity and spontaneous pain (assessed by novel place aversion), but the treatment had no effect on tactile hypersensitivity. Destruction of a defined population of spinal NK-1 receptor-expressing neurons, cells that respond to SP found in TRPV-1 afferents, blocked the neuropathic thermal and tactile hypersensitivity as well as spontaneous pain. Further manipulations revealed that the large-diameter dorsal column projection can mediate nerve injury-induced tactile hypersensitivity but does not contribute to spontaneous pain. This would suggest not only that different pathways from the periphery through the spinal cord convey different components of the neuropathic pain in this model but also that the therapeutic actions of agents acting through TRPV1 might be selective.

It is well known that both nerve and tissue injury produce hypersensitivity to evoked stimuli and ongoing, stimulus-independent pain. Using their validated approach based on pain relief elicits reward in nerve-injured rats, in a 4-day model of inflammatory pain, Okun et al. (2011) showed that the ongoing pain, driven by the peripheral site of injury (blocked by lidocaine applied here), is dependent on TRPV1-positive fiber, but the selective TRPV1 receptor antagonist AMG9810, although able to reverse the CFA-induced thermal hyperalgesia, had no effect at all on the CFA-induced ongoing pain or guarding behavior, suggesting that TRV1 receptor mechanisms do not contribute to spontaneous pain after inflammation. These novel approaches will shed much needed light on the processes that drive stimulus-evoked and stimulus-independent pains.

Because of the problems of the central effects of analgesics and the need for sustained pain control, there has been an increasing focus on development of new routes of drug administration to control persistent pain in a tolerable way and with acceptable patient compliance. Patches have been used for a wide variety of drugs—opioids for their central actions but at stable prolonged plasma levels or topical use of nonsteroidal anti-inflammatory agents and capsaicin where the aim is to target the local pain generators. Accordingly, recent applica-

tions of manipulation of TRPV1 function have been reported in terms of clinical application. These have been reviewed and vary from clinical trials using topical application in focal neuropathic pain states to explore the use of other agonists and also antagonists (Schumacher, 2010). A number of studies now employ the brief exposure to high-dose topical capsaicin in conjunction with prior application of a local anesthetic, required to transiently block the pain of exposure to this high level of the vanilloid. The use of resiniferatoxin, a potent TRPV1 agonist, is also being explored as an analgesic therapy. In contrast, the development of orally administered high-affinity TRPV1 antagonists is being used as a basis for production of effective analgesics.

Topical creams with capsaicin are used to treat pain from a wide range of chronic conditions, including neuropathic pain, and a *meta*-analysis of the data was published in 2009 (Derry et al., 2009). These approaches use a number of different doses of capsaicin applied to the skin and importantly, also considered the efficacy and tolerability of capsaicin for treating painful chronic neuropathies. In general, the indication is a localized neuropathic zone, such as that seen in postherpetic neuralgia and HIV-associated neuropathy. This excludes diabetic neuropathy, where the affected area, feet and lower legs, is often large. Here, only randomized controlled trials of least 6 weeks' duration, using topical capsaicin to treat neuropathic pain, were considered. In this type of study, because of the very nature of the therapy, the maintenance of blinding is an issue.

Six studies (389 participants) met the criteria for inclusion; in these studies, the patients received either low-dose (0.075%) capsaicin cream or placebo cream; the number needed to treat (NNT) for any pain relief over 6 to 8 weeks was 6.6. Two other studies (709 patients) looked at the use of high-dose capsaicin in the form of a single application of high dose (8%) capsaicin patch with placebo patch; the NNT for ≥30% pain relief over 12 weeks was 12. Here, the larger NNT may be complicated by the fact that the placebo patch actually contained the low dose, because they used 0.04% capsaicin as a control patch. It is notable that the duration of pain relief after a single application was prolonged but that the patch application needs care, because environmental spread of the capsaicin is a potential problem. Overall, in these studies, local skin reactions were more common with capsaicin but were tolerable and reduced with time (Derry et al., 2009).

Studies from Noto et al. (2009) highlighted that when high-dose capsaicin is used, there is a long duration of action of the therapy in that the pain relief is maintained for several weeks. Capsaicin is now thought, at high doses, to evoke a long-lasting but reversible refractory state in peripheral pain-sensing fibers involved in the induction and maintenance of neuropathic pain.

The targeting of nociceptive sensory nerve endings as a basis for the use of high-dose capsaicin patches in patients with neuropathic pain is based on preclinical observations mentioned previously, in which intradermal capsaicin caused a reduction in the density of epidermal fine nerve endings. The underlying mechanisms remain unclear. This pulling back of afferents, presumably producing a less-effective transduction of skin stimuli, has very recently been explored in humans. A high-concentration [8% (w/w)] capsaicin patch was applied to the thighs of healthy volunteers, and ENF density and the sensory changes produced were assessed by quantitative sensory testing. Thermal, tactile, and pinprick stimuli were used, which shed light on changes produced in C, Aβ, and Aδ fibers, respectively. These were followed for up to six months after capsaicin. At 1 week, there was an 80% reduction of ENF density, a modest 8% increase in tactile thresholds, and a reduction in sharp mechanical pain (decrease of approximately 15 percentage points) (Kennedy et al., 2010). Twelve weeks after capsaicin, ENF regeneration was seen with a return to normal sensory reports. It is noteworthy that at no time was any change seen in thermal testing, so heat- or cold-detection thresholds were unchanged, despite the common occurrence of short-lasting mild warming or burning sensations (Kennedy et al., 2010).

Turning to patient studies, peripheral neuropathy is a common neurological consequence of HIV-1 infection and often results in the development of chronic pain. A further problem is the neuropathy produced by antiretroviral treatment. The only published study in these patients was an open-label, 12-week pilot study of high-dose 8% capsaicin in patients with moderate-to-severe pain in both feet. Using a 60-min application, a 40% reduction in pain was produced. Approximately two thirds of patients in this small study ($n = 12$) responded with a pain decrease of greater than 30%, and others achieved a 50% reduction in pain, and treatment was generally well tolerated (Simpson et al., 2008). In March 2012, the food and drug administration did not approve the use of Qutenza (capsaicin) in HIV-associated neuropathy in the USA—despite the approval in the EU for treatment of nondiabetic localized peripheral neuropathic pain.

A controlled double blind study with more than 400 patients with postherpetic neuralgia compared a single 60-min application of 8% capsaicin with a 0.04% capsaicin control patch. Here the patients had a significantly greater mean reduction from baseline in pain during weeks 2 to 8 compared with the control group (32 versus 24%). A ≥30% reduction in pain was seen in 46 compared with 34% of control subjects, and the pain relief lasted for the entire 12-week study period (Irving et al., 2012). Most treatment-emergent adverse events were at the site of the patch, mainly erythema and pain, but described as transient and amenable to analgesics (Irving et al., 2012).

Thus, these studies suggest that a high-dose patch of capsaicin has tolerable efficacy in patients with a local-

ized pain as a result of nerve injury and that the modest reductions in pain might be clinically useful, especially because they last for months after application. Side effects are common but not problematic. This is explicable in terms of a "pulling back" of afferent fibers in the area of the patch. What is difficult to explain is the observation that heat and cold thresholds were unaltered, because clearly TRPV heat sensors are on fine fibers, as are TRPM8 cold receptors. This disconnect is also seen in the small but clear change in tactile sensitivity, because this modality is conveyed by large fibers that are not changed on visual inspection after high-dose capsaicin and do not possess TRPV1 receptors. These data may be explained by TRPV1 containing afferents driving central sensitization, so that loss of the ability of these fibers to be activated causes a "wind-down" of central responsivity. The ability of other modalities to access pain pathways centrally may be limited in the absence of central hypersensitivity.

So what other approaches might be possible? One obvious potential is the production of blockers of the TRPV1 receptor. A number of recent studies have been published on this topic. Based on the observation that patients with gastroesophageal reflux disease are hypersensitive to heat and acid, a TRPV1 antagonist (AZD1386) was tested on responses to painful heat, distension, electrical current, and acid in the esophagus in healthy volunteers (Krarup et al., 2011). It is noteworthy that both heat and pressure pain applied on the arm was used to gauge possible effects on somatic stimuli. Almost all of the 21 participants completed the protocol. In the esophagus, AZD1386 increased pain thresholds to heat stimuli by 23 to 28%, depending on the dose. The drug also increased skin heat tolerance, and the effects on thermal stimuli (which were selective, in that the mechanical pain tests were unaltered) lasted for 2.5 h. Half of the subjects reported "feeling cold" and body temperature was elevated by 0.4 to 0.7°C, depending on the dose; however, no other severe adverse-event problems were reported (Krarup et al., 2011).

Another study in healthy volunteers used the TRPV1 antagonist (R)-(5-tert-butyl-2,3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)-urea (ABT-102) with proven efficacy in a number of preclinical pain models. Here, the sensory effects with somatic stimuli were tested, as was the potential of drug-induced thermosensory impairment. ABT-102 was tested in a three-dose, double-blind, placebo-controlled, randomized trial. The drug caused significant dose-dependent increases in the heat pain threshold and reduced pain scores for suprathreshold heat. These were seen over the weeklong experimental duration and were present for both oral and cutaneous stimuli with selectivity, in that cutaneous cold detection did not alter. All effects were fully reversed by day 10, and no safety issues were seen; body temperature stayed below 39°C in all subjects. Thus, in this randomized controlled trial, this selective TRPV1 antagonist ABT-102 potently and reversibly increased the threshold for

heat pain but also reduced the pain from suprathreshold oral and cutaneous heat. Thus, this study adds to the previous data, suggesting that these antagonists are both effective and tolerable (Rowbotham et al., 2011).

Thus far, based on its distribution and functions, TRPV1 can be considered a highly suitable target for developing small-molecule antagonists, although the alterations in body temperature could lead to the threat of abandonment of such antagonists. Furthermore, as mentioned previously, this receptor is not found only in neurons, so blockade could also be problematic. Some of these issues have been reviewed by Premkumar and Bishnoi (2011), but the thermal changes as a potential problem were noted several years ago.

In one sense, it is reassuring that, in terms of translation from animals to the clinic, in all species tested (except birds—thus a chicken jalfrezzi is a crime against nature, but still rather tasty), the agonists at TRPV1 cause pain and hypothermia—when delivered systemically; thus, not surprisingly, several of the various antagonists of TRPV1 block pain in rat and mouse models of inflammation, OA, and cancer-induced pain. But, from rodents to primates, body temperature also increases. This ancient conserved function of TRPV1 in body-temperature maintenance was a hurdle for clinical development of one antagonist, N-(4-(6-(4-trifluoromethylphenyl)pyrimidin-4-yloxy)benzothiazol-2-yl)acetamide (AMG 517) (Gavva et al., 2008).

However, several other TRPV1 antagonists are currently being evaluated, and a very recently published article sheds light on the problem of antagonist-induced hyperthermia while developing therapeutic agents (Watabiki, 2011). With several new antagonists producing the same elevation of body temperature—is this a class effect or not? The TRPV1 receptor is activated by a variety of stimuli, such as endogenous ligands and low pH. This range may be important in terms of hyperthermia in animals and humans.

One important issue is that TRPV1 antagonists differentially block three modes of TRPV1 activation: by heat, protons, and chemical ligands (e.g., capsaicin). A study compared potencies in these three modes of TRPV1 activation with the potency of a TRPV1 antagonist to cause hyperthermia in rats, mice, and guinea pigs using eight TRPV1 antagonists with different pharmacological profiles. With the use of mathematical modeling, it became clear that the hyperthermic effect is highly related to the extent of TRPV1 blockade in the proton mode with no to moderate sensitivity in the capsaicin mode and no sensitivity in the heat mode. Thus, hyperthermia-free TRPV1 antagonists that do not block TRPV1 activation by protons and have low potency to block the capsaicin mode may be of use, but are these types of compounds still effective?

The TRPV1 antagonist N-(1-methyl-2-oxo-1,2,3,4-tetrahydro-7-quinolyl)-2-((2-methylpyrrolidin-1-yl)methyl)biphenyl-4-carboxamide (AS1928370) binds to the res-

iniferatoxin-binding site on TRPV1 and so, as expected, will reduce capsaicin-mediated inward currents. AS1928370 also inhibits the capsaicin-induced Ca^{2+} flux in human and rat TRPV1-expressing cells yet has a very small effect on the proton-induced flux (Watabiki et al., 2011). AS1928370 attenuated capsaicin-induced secondary hyperalgesia and mechanical allodynia after neuropathy and was effective on inflammatory pain. AS1928370 had no effect on body temperature up to 10 mg/kg p.o. but had a hypothermic effect at higher doses.

XV. Conclusion

Capsaicin administered topically, intradermally, or orally has proven to produce complex effects but remains a reliable and reproducible way to investigate peripheral and central mechanisms underlying certain hypersensitivities. In addition, we have discussed how it may also be a useful treatment of certain pain states, including neuropathies.

In animal models, capsaicin has been used extensively to elucidate the molecular mechanisms that underpin central sensitization as well as investigating potential pharmacological interventions. In human models, it has been shown to evoke similar signs and symptoms of central sensitization, including hyperalgesia and allodynia. This suggests that similar mechanisms are involved in changes of pain processing after capsaicin administration in both animals and humans. Further characterization of capsaicin as a tool and its use as a reliable translational model, using similar evoked responses and time frames, with consideration of the dose, would be useful to confirm this translation. However, from this review we conclude that agents acting on TRPV1 receptors, as well as capsaicin itself, will still generate plenty of interest in the future and will shed light on pain mechanisms. There is plenty of excitement to come with this vanilloid.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: O'Neill, Brock, Estrup Olesen, Andresen, Nilsson, and Dickenson.

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