ELSEVIER

Life Sciences, Vol. 60, No. 10, pp. 681-696, 1997

0024-3205/97 $17.00 + .00

PII S0024-3205(96)00567-X

MINIREVIEW

EUPHORBIUM: MODERN RESEARCH ON ITS ACTIVE PRINCIPLE, RESINIFERATOXIN, REVIVES AN ANCIENT MEDICINE

Giovanni Appendino[1] and Arpad Szallasi[2]

[1]Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, via Giuria 9, 10125 Torino, Italy and
[2]Department of Anatomy & Neurobiology, Washington University School of Medicine, Campus Box 8108, 660 S. Euclid Ave, St.Louis, MO 63110

(Received in final form December 13, 1996)

Summary

Resiniferatoxin, an ultrapotent capsaicin analog present in the latex of *Euphorbia resinifera*, interacts at a specific membrane recognition site (referred to as the vanilloid receptor), expressed by primary sensory neurons mediating pain perception as well as neurogenic inflammation. Desensitization to resiniferatoxin is a promising approach to mitigate neuropathic pain and other pathological conditions in which sensory neuropeptides released from capsaicin-sensitive neurons play a crucial role. Clinical trials to evaluate the potential of topical resiniferatoxin treatment to relieve pain associated with diabetic polyneuropathy and postherpetic neuralgia are in progress. Though resiniferatoxin was isolated only two decades ago, the dried latex of *Euphorbia resinifera*, called Euphorbium, has been in medicinal use since the time of recorded history. This review highlights the most important events in the history of this ancient medicine, from the first written record of the therapeutic potential of Euphorbium (at the time of the reign of the Roman Emperor Augustus) to the identification of its active principle as resiniferatoxin in 1975. A brief overview of the enormous contribution of resiniferatoxin to our current understanding of the anatomical localization, function, and pharmacology of vanilloid receptors is provided. Lastly, the mechanisms are summarized by which capsaicin and resiniferatoxin, despite sharing receptors, may have dissimilar biological actions.

Key Words: euphorbium, resiniferatoxin, capsaicin, vanilloid receptors

Foreword: Nicholas (Miklós) Jancsó (1903-1966), the brilliant Hungarian pharmacologist, who almost single-handedly transformed capsaicin from a pharmacological curiosity to a widely used neuropharmacological tool and who was the first to postulate a receptor for capsaicin, died 30

years ago. With this review, we wish to pay tribute to him for his enormous contribution to the vanilloid field. This review is also dedicated to the memory of his student, Ferenc Joó, who died prematurely this year, for his invaluable help in proving that resiniferatoxin functions as a capsaicin analog.

Introduction

Patients suffering from diabetic neuropathy who participate in the ongoing clinical trials with resiniferatoxin (RTX), an ultrapotent capsaicin analog, are hardly aware of the fact that they are receiving a remedy more than 2000 years old. RTX is the active ingredient in Euphorbium, a drastic plant resin known since the Roman times. Euphorbium is obtained from *Euphorbia resinifera* Berg, a large, leafless cactus-like perennial (1). The plant, a native of the Anti-Atlas Mountains of Morocco, grows from 4 to 6 feet high and has a thick woody stem giving rise to a number of very fleshy, quadrangular, spiny green branches (1). The flowers are small and yellowish; they appear in groups of three at the top of the branches. The fruit is a small capsule. *E. resinifera* belongs to the Euphorbiaceae (commonly known as the Spurge family), one of the most important medicinal families of plants (2). Euphorbiaceae comprise more than 200 genera and 3000 species, distributed over almost the entire Earth, which renders it one of the largest families of flowering plants (2). All euphorbias contain an irritant latex and a great number of them have been used for medicinal purposes, mainly as a purgant or to remove warts and freckles. Despite the dangerous nature of the latex, many euphorbias are commonly cultivated as indoor ornamentals for their conspicuously colored leaves and flowers. Notable species include *E. pulcherrima* Willdt (better known as Poinsettia), a popular Christmas plant in the United States, and the spiny Christ's-thorn, *E. milii* Desmoul. *E. resinifera* itself, despite its irritancy and prickly stems, is a popular house plant in Europe, though cultivated samples rarely grow higher than 2 feet.

History of the Medicinal Use of Euphorbium from Antiquity to the Present

E. resinifera is laden with a milky juice (latex) which has been collected by the inhabitants of Morocco from very remote times by wounding the stems and collecting the exuded, dried resin "tears" (1). Tradition has it that it was the Roman physician Euphorbius (not to be confused with Euphorbos, the Trojan warrior of the *Iliad*), who first used Euphorbium to treat the arthritic pains of the Emperor Augustus. This theory, however, is not corroborated by any written ancient sources, and probably derives from a confusion of Euphorbius with his brother, Antonius Musa, who in fact used an infusion of lettuce (*sic !*) and cold baths to heal the Emperor, and was honored in return by a statue in the temple of Aesculapius, the shrine of Roman medicine (see below) (3) As a curiosity, it is worth mentioning that Romans did not eat lettuce because of its alleged anaphrodisiac properties, still mentioned by the great botanists Andreas Lobelius (XVIth century) and Carl Linné (XVIIIth century).

Probably it is King Juba II of Mauretania (50 BC - 23 AD) instead who deserves credit for discovering the medicinal potential of the resin, which he described in a lost treatise, entitled Περι οπου (On Latex) (4). This book is considered to be the first pharmacological monograph (5). Juba II was the son of Juba I, the king of Numidia (presently Eastern Algeria), one of the fiercest foes of Julius Caesar, who committed suicide after his defeat at the battle of Thapsus (47 BC) (6). Caesar brought the son of his defeated enemy to Rome, where the child was paraded in his triumph (along with Vercingetorix and other defeated enemies), and was later brought up as a loyal Roman citizen. He was made the king of Mauretania by Augustus, and later he married Selene, the daughter of Antony and Cleopatra (6). He is better known as a man of letters than a king: for example, Pliny

the Elder mentiones Juba II as a major source of his monumental *Naturalis Historia* (7). According to Pliny (7), Juba II named the material Euphorbium in honour of his physician Euphorbius. If so, he chose an evocative name, as Euphorbius in Greek means "well-fed", and thus Euphorbia may also refer to the fattened look of the plant. According to another theory, Euphorbium should rather be translated as "good fodder", since famished camels occasionally eat cactus-like succulents. Though black rhinos, in fact, browse the branches of *E. tirucalli* L. (2), this interpretation is highly unlikely, unless camels are connoisseurs of hot, spicy plants or, alternatively, they lack vanilloid (resiniferatoxin) receptors. Despite these uncertanties, there appears to be a consensus among scholars that the resin was named by Juba II after Euphorbius. As the famous statement credited to Carl Linné reads (quoted in 8): "*Ubi jam Musae statua? Periit! Evanuit! Euphorbii autem perdurat, nec unquam destrui potest.*" - i.e. whereas the statue of Musa (see above) crumbled, the name of his brother, Euphorbius, will live forever in the name of a drug. Ironically, nowadays Euphorbius is better known as the father of balneotherapy, a practice immortalized by Oratius, than as the godfather of Euphorbium.

Only a few fragments of King Juba's treatise are left (6), thus information on the medicinal use of Euphorbium in this period is rather scanty. Subsequently, Euphorbium is mentioned both in the Greek (e.g. Dioscorides) and Latin (e.g. Pliny the Elder) medical literature as a sternutative (nose irritation) as well as vesicant (skin irritation) agent (9). It was also used in the treatment of lethargy: patients could be awakened by "touching their nostrils with a solution of Euphorbium in vinegar" (10), with dramatic results, no doubt about it. Other uses of Euphorbium, mentioned by Pliny (7), are, however, puzzling, such as the instillation of Euphorbium solutions into the eyes to sharpen sight, or its generalized use against poisons and snake bites. In this case the cure sounds almost worse than the disease: according to Pliny (7), no matter where the bite is, an incision is to be made on the skull and the medicament should be inserted there!

However, it is not unlikely that during Antiquity, as well as the Medieval Ages, a variety of by now obsolete, irritant remedies were collectively referred to as Euphorbium. For example, Dioscorides himself in his *De Materia Medica* (11) described the source of Euphorbium as "a ferula", possibly confusing it with *Thapsia garganica* L., an umbelliferous species widespread in the Southern Mediterranean region, from the latex of which the likewise very irritant compound thapsigargin can be isolated (12). The probably oldest known drawing of the plant (in an Arabic version of *De Materia Medica* by Dioscorides, dated 1083) (13) is also erroneous, as it depicts a ferula, but a later manuscript (1244) already correctly represents it as a thorny cactus (14). As Morocco was never fully integrated into the Roman Empire, it is unlikely that educated Latin writers could have visited its interior where Euphorbium was produced. Nor could Christian scholars visit there during the Islamic Conquest. No wonder that the botanical origin of Euphorbium had been the subject of heated scientific dispute for centuries. As a rare example of honesty, Matthiolus in his *Pedacio Dioscorides* (1544), the Goodman & Gilman of the Renaissance, did not include any drawing of the plant source of Euphorbium that he had admittedly never seen ("*La pianta che produce l'Euforbio non vidi già io mai in tempo di vita mea*"; i.e. never in my life have I seen the plant source of Euphorbium) (15).

During the Renaissance, Euphorbium was widely used as a sternutatory (to provoke sneezing), until its popularity was overshadowed by tobacco (5). However, it remained a standard constituent in vesicant plasters, the most (in)famous of which are the "*Mosche di Milano*" (*Emplastrum Cantharidum perpetuum*) and the "Emplastre des Capucines". The "Flies of Milano" (16) was a mixture of Spanish flies and Euphorbium (10 parts each), in turpentine, colophony (90 parts each) and Storax (10 parts). The formula of the plaster of the Capucins is no less amazing (17): wax (90 parts), terebinde, Ammoniacum, Olibanum, mastic (30 parts each), Euphorbium and Pyrethrum (60 parts each). Furthermore, Euphorbium was frequently used in purgatives, although this prac-

FIG. 1

The collection of the latex of an euphorbia as depicted in Codex Ayasofia (3703, f. 136.1.3, Freer Gallery of Art, Smithsonian Institution, Washington, DC), and Arabic Version of *De Materia Medica* by Dioscorides.

tice was strongly disapproved by most physicians on account of the severity of its action. As summarized by Étienne-François Geoffroy in his "Traité de Matière Médicale" (1743), the first systematic treatise of pharmacognosy, "Il ne faut pas employer ce purgatif, ou du moins il ne faut seulement l'employer dans le maladies dans lesquelles les membranes des viscéres sont attaquées de paralyse." (i.e., this purgative should not be used at all or it should only be used in diseases in which the viscera are already paralyzed) (18). Interestingly, 180 years later the 3rd edition of the Merck's Index still lists Euphorbium as a drastic purgative (19); the 4th edition only notes its use as a vesicant in veterinary practice (20); but Euphorbium is completely missing from the subsequent editions.

The first medical use of Euphorbium that with hindsight can be considered as scientifically solid was its direct application on cavities or nerves to suppress chronic pain. As noted by Geoffroy: "On vante l'usage de l'Euphorbe comme excellent dans la carie des os et la piqûure des nerfs" (i.e., Euphorbium is reported to be an excellent treatment for bone cavities and nerve pains) (18). In Transsylvania, it was a popular and effective remedy to mitigate tooth ache (21). It is perplexing why this powerful analgesic drug had vanished later from medical practice. Euphorbium injected intraarticularly was also succesfully used until recently in the treatment of otherwise untractable bone and articular tuberculosis (22).

The irritancy of Euphorbium was legendary. Matthiolus in his above mentioned *Pedacio Dioscorides* reports that pharmacists refused to pulverize it, leaving this task to "facchini ò altre persone vili et mecaniche" (that is, to blue collar workers, in today's lingo) (15). Two hundred years later, powdering Euphorbium was still left to "paysans ou gens de basse condition" (i.e., to

peasants and other folks of low social standing) (18). No wonder that Euphorbium soon enjoyed a sinister fame among the makers of practical jokes. For example, balls were disrupted by the general sneezing that followed the spreading of Euphorbium powder on the floor (23). Such (mis)use of Euphorbium even found its way into the dramatic literature: Panurge, the merry and cowardly companion of Pantagruel in Rabelais' play, entitled "Gargantua et Pantagruel", makes fun of a young girl by giving her a beautiful handkerchief sprinkled with Euphorbium powder. The effect is quite dramatic, to Panurge's highest delight, the poor victim sneezes "quatre heures sans repos" (i.e., four hours without rest).

One would expect that such a powerful irritant should have attracted the attention of pharmacologists. Therefore it is a mystery why Euphorbium had completely been ignored when other irritants such as tobacco became the focus of attention. Maybe pharmacologists found Euphorbium simply too toxic to work with (see above).

By the beginning of the nineteenth century Euphorbium had lost its importance as a medicine but it still retained its industrial uses (antifouling agent, leather softening, etc) (23). The colonisation of Northern Africa ended the monopoly of Arab merchants in the Euphorbium trade. In 1809 Jackson, an English merchant who had spent several years in Morocco, published the first "eye-witness" drawing of the plant in his "Account of the Empire of Morocco and the district of Suse" (24). Ironically, Jackson probably confused *E. resinifera* with *E. beaumeriana* Coss, and it was shortly realized thereafter that the fragments of the plant present in commercial Euphorbium did not fit the drawing in Jackson's book (25). In 1863, the German botanist Berg attempted the characterization of the plant source of Euphorbium based on the fragments recovered from commercial material. He realized that the fragments must have come from a new, yet undescribed, species of Euphorbia, which he tentatively named *E. resinifera* (26) Seven years later live plant specimens shipped to Kew Gardens in England by the British vice-consul in Morocco confirmed Berg's conclusions (quoted in 25).

Before World War II, *E. resinifera* attracted considerable attention as a fuel source (2). New cultivations were established by the Fench in Morocco, which were later abandoned when the War broke out. Not unexpectedly, the oil crisis in the seventies rekindled interest in the potential use of Euphorbias as a gas substitute, though these later studies focused on a less irritant species, *E. lathyris* L., instead (27).

Nowadays Euphorbium is still obtained in the traditional way, described by Pliny almost two millennia ago: "*Vis tanata est, ut e longinquo sucus excipiatur incisa conto; subitur exapulis ventriculo haedino. umor lactis videtur deflure; siccatus cum coiit, turis effigem habet*" (i.e. Its potency is such that the juice, obtained by incision with a pole, is gathered from a distance. It is caught in a container made of a young goat's stomach. It is like milk when it drops, but, once dried, looks like frankincense) (8). Euphorbium is collected in late Summer, and is mainly exported from Mogador. Even in the thirties, more than 100 tons of Euphorbium were shipped to Europe annually (1). More recent reports on the commercial production of Euphorbium are not available; nonetheless, its export to Europe must have decreased very substantially, as scientists in the seventies experienced great difficulties in obtaining the latex. For example, when Peter M. Blumberg, at that time a professor at Harvard University, became interested in the mechanism of action of resiniferatoxin, the active principle in Euphorbium, he had to request the intervention of Senator Edward Kennedy so that the American Consulate in Morocco could collect Euphorbium for his studies.

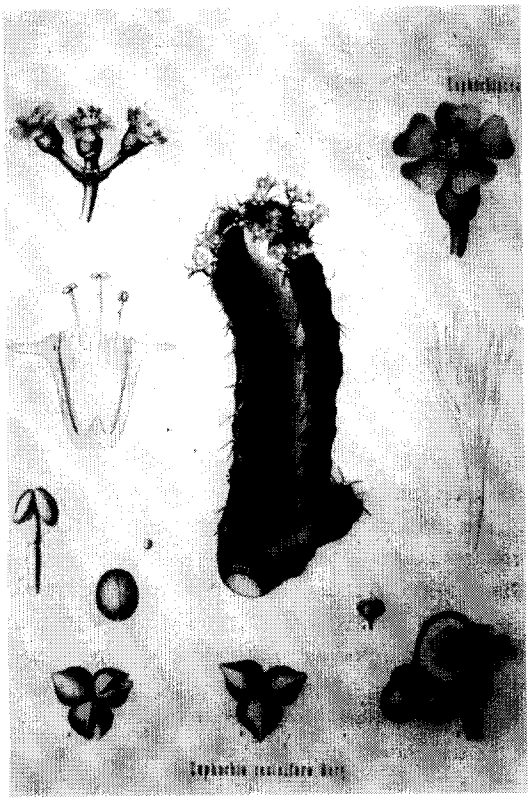

FIG. 2

A century old depiction of *Euphorbia resinifera* Berg in Köhler's book, entitled "Medizinal Pflanzen in naturgetreuen Abbildungen mit kurz erlauterndem Texte", published by Gera-Untermhaus in 1883.

Identification of the Active Principle in Euphorbium as Resiniferatoxin

In 1975 Hecker's group in Germany isolated the active principle in Euphorbium and named it resiniferatoxin (RTX) (28). During the drying process, the concentration of RTX in the latex diminishes due to oxidation (29), which might explain why "young" samples of Euphorbium were not considered suitable for medicinal purposes. As noted by Matthiolus, "*Quello che non passa un anno, per la sua molta attività non è da usare*" (i.e., samples less than one year old should not be used because they are too powerful) (15). Furthermore, the latex of *E. resinifera* also contains other irritant substances with distinct mechanism of action (see below), the stability of which might be different from that of RTX. With hindsight, in addition to its irritancy, this variability in chemical composition might have been the principle reason for the lack of enthusiasm for Euphorbium among modern day pharmacologists.

In addition to RTX, Euphorbium also contains ingenol- and 12-deoxyphorbol (but not phorbol) esters (30). These compounds are of special importance, both as tumor promoters and selective ligands for their receptor, the enzyme protein kinase C (PKC) (31). Euphorbium is also a good source of 12-deoxyphorbol, the 13-acetate derivative of which (prostratin) is an important lead compound with anti-HIV activity (32). Tumor promoting phorbol esters and RTX are structurally related with the notable difference that whereas a free OH group at the C20 position is essential for phorbol ester-like activity, RTX, by contrast, is esterified with homovanillic acid at this critical

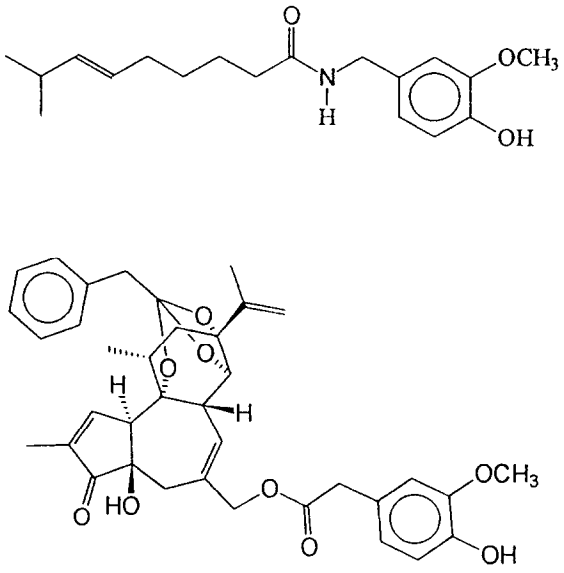

FIG. 3

Structures of capsaicin (above) and resiniferatoxin (below).

position (33). Nonetheless, at the time when RTX was first isolated, the primary target for phorbol esters was not known, and the initial interest in RTX was generated by the search for phorbol ester receptors. RTX, however, failed to induce the cellular responses characteristic of typical phorbol esters (34), and did not compete effectively for specific phorbol ester binding sites either (35). Even more important, RTX turned out not to promote tumor formation (36).

Resiniferatoxin Functions as an Ultrapotent Capsaicin Analog (Vanilloid) with a Distinct Spectrum of Biological Activity

The real turning point in the two thousand years old history of Euphorbium was the discovery that resiniferatoxin functions as an ultrapotent analog of capsaicin, the pungent principle in hot pepper (37). This exciting story has recently been reviewed extensively (33,38,39). Here it suffices to mention that capsaicin is pungent by selectively activating a subset of primary sensory neurons, with cell bodies (predominantly of the small size) in dorsal root, trigeminal as well as nodose ganglia (see 40 for extensive review). These capsaicin-sensitive neurons give rise to two unmyelinated C-fibers (often referred to as a pseudounipolar structure); the afferent fiber conveys nociceptive information into the central nervous system (where fibers terminate in the dorsal horn of the spinal cord), whereas the peripheral terminals are the sites of release for a variety of proinflammatory neuropeptides, of which calcitonin gene-relate peptide (CGRP) and the tachykinin substance P (SP) are the best known examples (40). Excitation of sensitive neurons by capsaicin is followed by a lasting refractory state, traditionally termed desensitization (41), or, under certain conditions such as neonatal treatment, gross neurotoxicity (42). We owe the gamut of our present knowledge regarding the biological actions of capsaicin to the pioneering studies of the late Nicholas (Miklós) Jancsó (1903-1966), who not only predicted the existence of sensory neuropeptides (in his words "neurohumors") in capsaicin-sensitive neurons, but also postulated a receptor for capsaicin (currently known as the 'vanilloid' receptor; see below).

Generally speaking, RTX acts as an ultrapotent capsaicin analog, that is, it mimics most biological responses charasterictic of capsaicin with a hundred to several thousand-fold higher potency (33,38,43). However, RTX and capsaicin also show striking differences in relative affinities for a variety of biological end-points studied. A notable example of this phenomenon was reported by Maggi and coworkers who found RTX several thousand-fold more potent than capsaicin for desensitizing the contractions of the isolated rat urinary bladder, but only similar in potency to capsaicin for contracting the isolated rat urinary bladder (44). In most cases when RTX and capsaicin differ in potency the situation is such that RTX preferentially causes desensitization whilst using capsaicin excitation dominates (43). Recent findings might provide a rational to explain this interesting divergence between RTX and capsaicin actions. However, first it has to be summarized what is known at present about the pharmacology of vanilloid receptors.

Vanilloid Receptors: Emerging Evidence Suggests a Receptor Family with Members of Differing Pharmacology

As RTX and capsaicin structural analogs (resiniferanoids and capsaicinoids) share a (homo)vanillyl moiety as a stuctural motif essential for bioactivity, but differ substantially in the rest of the molecule, these compounds together appear to be best described as 'vanilloids'. In keeping with this terminology, the receptor at which they interact may be referred to as the 'vanilloid' receptor. However, by the time this new terminology had gained acceptance in the literature, it turned out that the ligand recognition selectivity of vanilloid receptors is much broader than anticipated. For example, irritant sesquiterpenoids possessing an unsaturated 1,4-dialdehyde moiety (the paradigm of which is isovelleral, isolated from the mushroom *Lactarius vellereus*) are pungent by activating capsaicin-sensitive neurons in a vanilloid receptor-mediated fashion (45). Other natural products structurally unrelated to capsaicin, such as scutigeral, a triprenyl phenol isolated from the edible mushroom *Albatrellus ovinus* , or cinnamodial, an irritant sesquiterpenoid found in the East African plant *Warburgia ugandensis* , are also able to inhibit specific RTX binding to rat spinal cord preparations (O. Sterner, G. Vidari, M.R. Witt, and A. Szallasi, unpublished observations). Of course, from a chemical point of view, these recently identified ligands for the vanilloid receptor cannot be called vanilloids, which, in turn, questions the validity of the term 'vanilloid' receptor. Receptors are preferentially named after their endogenous activators. We are optimistic that such endogenous activators of 'vanilloid' receptors will shortly be identified, and thus the proper taxonomy for 'vanilloid' receptors and their ligands resolved. Until then, we propose that the term 'vanilloid' receptor remain in use, since it is still a much broader term than the alternatively suggested capsaicin receptor.

Specific binding of [^{3}H]RTX provided the first direct proof for the existence of vanilloid receptors (46). Additional evidence was furnished by the development of a competitive vanilloid receptor antagonist, called capsazepine (47). Most recently, an autoradiographic approach has been developed to visualize specific [^{3}H]RTX binding sites (vanilloid receptors) in several species, including man (39). As indicated by previous functional studies (summarized in 40), vanilloid receptors are present throughout the entire length of capsaicin-sensitive primary sensory neurons (39,48). In addition, there is functional evidence implying the existence of capsaicin-sensitive intrinsic neurons in the brain (reviewed in 40). Interestingly, such neurons do not show up on rat brain sections subjected to [^{3}H]RTX autoradiography (49), implying that these neurons, if they exist, express low density and/or low affinity vanilloid receptors.

Vanilloids show diverse biological activities which is difficult to reconcile with the model of a single, shared receptor. Therefore it was proposed in 1989 that vanilloid receptor subclasses with differing pharmacology exist (37). Although this heterogeneity of vanilloid receptors remains

putative until the actual existence of such receptor subclasses is confirmed by molecular cloning, presently at least three lines of strong, albeit indirect, evidence argue for a vanilloid receptor family:

1) patch-clamped sensory (dorsal root as well as trigeminal) ganglia neurons display an amazing heterogeneity of vanilloid-induced inward ion currents (capsaicin-induced currents are typically rapid in onset and then follow a bi-phasic kinetics, with an initial partial recovery and a subsequent steady-state phase, whereas usually RTX evokes slow and lasting currents, which are heterogenous in the magnitude of their peak) (50);
2) cultured dorsal root ganglia neurons show strikingly different structure-activity relations for binding and calcium uptake, respectively, suggesting that these assays detect distinct vanilloid receptor subclasses (51); and
3) in the perfused rat hindlimb, where vanilloids induce a bi-phasic oxygen uptake response (an initial increase followed by a depression to levels below baseline, with the second phase being coupled to vasoconstriction), pharmacological evidence implies the existence of two peripheral vanilloid receptor subclasses, the so-called VN_1 mediating oxygen uptake (which recognizes vanilloids as well as the competitive vanilloid receptor antagonist capsazepine with high affinity), and the putative VN_2, responsible for vasoconstriction (which binds vanilloids with low affinity, is not blocked by capsazepine, but is particularly sensitive to the so-called functional vanilloid receptor antagonist ruthenium red) (52).

Systemic capsaicin activates a variety of cardiovascular as well as respiratory reflex responses (e.g. decreased heart rate, low blood pressure, and shallow breathing) which, in turn, make the therapeutic window of capsaicin very narrow. Experimental therapeutic use of RTX, though less complicated by such reflex responses, is not devoid of undesired side-effects either. For example, RTX at doses at which it desensitizes against pain perception also causes a profound drop in body temperature (37). An important implication of the existence of vanilloid receptor subclasses mediating different biological responses is the possibility that receptor subtype selective vanilloids may be synthesized, which, for example, do not affect body temperature if they are intended to supress pain perception only, but do reduce body temperature when they are meant to be antifebrile as antiinflammatory drugs. There is preliminary evidence that such improved vanilloids can in fact be synthesized. Notable examples include olvanil and nuvanil, which apparently do not activate peripheral vanilloid receptors (53), as well as certain phorbol-, and 12-deoxyphorbol-based 20-homovanillates, which fail to induce a measurable hypothermia response at doses at which they effectively desensitize against neurogenic inflammation (54).

Last but not least, vanilloid receptors show marked species-related differences (see 38 for review). It is important to keep in mind that the gamut of our present knowledge regarding the pharmacology of vanilloid receptors was obtained in the rat, and does not necessarily apply to human vanilloid receptors. Nonetheless, the major features of vanilloid receptors (high affinity RTX binding; positive binding cooperativity; sensitivity to protons; recognition of neuroleptics, etc.) appear to be similar in rat and human tissues.

Vanilloid Receptors: Receptor Structure and Effects of Activation

There is general consensus in the literature that vanilloids possess a specific membrane recognition site (receptor) which is either a non-selective, ligand-gated cation channel *per se* or at least is closely connected to one (55,56). Vanilloid receptors are expressed almost exclusively by small-diameter sensory neurons giving rise to unmyelinated C-fibers. Notable exceptions include sen-

sory neurons of nodose ganglia (39,57) as well as some, rather loosely defined intrinsic neurons in the brain. Within the latter group, anterior hypothalamic neurons are believed to mediate the effects of vanilloids on body temperature regulation (58). The relevance of other brain neuron subsets, which have been implicated in capsaicin actions, is either unclear (e.g. enhanced glucose utilization in a number of brain areas other than the hypothalamus following capsaicin treatment) (59) or questionable (profound neurodegeneration throughout the neuroaxis of the rat given high doses of capsaicin) (60), as capsaicin is known to interact non-specifically with both proteins (receptors and enzymes) and lipids (affecting membrane fluidity, which, in turn, can modify protein functions) (see 40 for details).

Receptors containing intrinsic ion channels are frequently multisubunit. The radiation inactivation size of vanilloid receptors (approximately 300 kDa in the pig) is consistent with an oligomeric structure (61), as is the positive cooperative binding behaviour that vanilloid receptors display (33,38,39,48). Both receptor binding (38,39) and resulting channel opening (62) can be fitted to the allosteric Hill equation with a cooperativity index of 2, implying the existence of at least 2 interacting binding sites. The biological role for this positive cooperative vanilloid receptor function is essentially unknown, but in case of other receptors positive cooperativity is assumed to serve as an amplificatory mechanism to enhance the efficacy of endogenous ligands produced in low quantities (63). Thus, positive cooperativity of vanilloid receptors can be interpreted as an argument for the existence of as yet unidentified endogenous vanilloids. However, vanilloid receptors apparently bind different ligands with different degrees of cooperativity, suggesting that this binding behaviour is a ligand-induced feature rather than an inherent property of vanilloid receptors (54,64).

In addition to at least two vanilloid binding domains (presumably located in the inner side of the membrane) and the channel pore, vanilloid receptors seem to have allosteric modulatory sites as well. First, there appears to be a protonation site, the occupation of which interferes with RTX binding (65) and leads to channel opening (55,66) and, second, there is evidence for another regulatory site recognizing neuroleptic drugs, which modulates (either enhances or blocks) both vanilloid binding and the resulting calcium influx, but is apparently unable to activate the channel in the absence of vanilloid agonists (67,68). The protonation site has attracted much attention lately as a possible mediator of pain associated with inflammation and myocardial infarction (66). There are discrepancies in the literature regarding the connection between capsaicin-, and proton-sensitivity, inasmuch as conductances activated by capsaicin and protons overlap only partially. However, these apparently conflicting results can easily be resolved by postulating that, on the one hand, not all vanilloid receptors have protonation sites (another form of vanilloid receptor heterogeneity) and, on the other hand, such protonation sites may also exist independent of vanilloid receptors. Less clear is the relevance of the site which binds phenothiazines and other neuroleptic drugs. Since neuroleptics are often used in clinical practice to ameliorate neuropathic pain on an empirical basis, an attractive hypothesis to explore is that neuroleptics may suppress chemical transmission in the spinal cord by enhancing the efficacy of putative endogenous vanilloids in such a way that they not only excite but also desensitize central terminals of vanilloid-sensitive neurons.

Agonist binding to vanilloid receptors leads to channel opening and subsequent mono- and divalent cation, predominantly calcium, influx. The so-called functional vanilloid antagonist, ruthenium red, is thought to block the channel pore itself (69). Tetrodotoxin-sensitive, fast sodium channels are activated, and the resulting action potentials are perceived as pain in the CNS (55,56). As intracellular calcium levels are rising, voltage-sensitive calcium channels are first activated, leading to transmitter release, and then inhibited, blocking the very same response (70). Calcium also activates a variety of enzymes, including PKC (71) and calcineurin (72). As a counterregula-

tory mechanism, neurons try to sequester excess calcium into intracellular depots, most notably to the mitochondria. However, as mitochondria become overloaded with calcium, the mitochondrial respiratory chain gets impaired and the overstimulated neuron may ultimately perish, especially when vanilloids are given to neonates (73). An auxiliary mechanism of neurotoxicity is the osmotic damage that follows sodium influx (and subsequent NaCl formation) through the vanilloid receptor-linked channel (55).

How Can Vanilloids Relieve Pain?

Broadly speaking, vanilloids may block pain perception by two basic mechanisms: (1) vanilloids can make the sensitive neurons non-functional either by desensitization or neurotoxicity (direct action), or (2) vanilloids may suppress the processing of nociceptive information by second-order neurons in the spinal dorsal horn (indirect mechanism). It is self-explanatory that whereas in the first case vanilloids are capable of blocking those pain modalities only which are mediated by vanilloid-sensitive nerves (e.g. thermal and chemogenic pain), in the second scenario vanilloids may also inhibit the processing of information supplied by non-vanilloid-sensitive nerves. Whilst it has repeatedly been confirmed that neurons desensitized to vanilloids do not transmit nociceptive information, at present it is not clear to what extent vanilloid treatment may alter the function of second-order neurons.

Desensitization itself is a presumably complex and, as yet, poorly understood process, in which different stages might be distinguished (74). Receptor-level desensitization may, of course, protect against vanilloid-induced pain only, and is of little importance from a therapeutic point of view. Nevertheless, in this context it is worthwhile to mention that vanilloid receptors have been suggested to cycle between closed and open states, via intermediate states reflecting receptor-level desensitization (50). According to this model, tachyphylaxis reflects the time needed for the receptors to recover from the desensitized states to the basic (closed) state. Strictly speaking, during tachyphylaxis neurons do not respond to subsequent vanilloid administrations (since their vanilloid receptors are already occupied and are in a resting state), but are still excited by stimuli which act on other targets. Such 'pure' tachyphylaxis, however, is seldom observed (unless using *in vitro* preparations in a calcium-free environment) since the calcium influx, which follows receptor activation, alters the function of the whole neuron. Among the calcium-activated processes, the block of voltage-sensitive calcium channels (70) as well as the activation of the enzyme calcineurin (72) are believed to play a pivotal role in desensitization. Regardless of the underlying subcellular mechanisms, desensitization to vanilloids inevitably leads to an inhibition of neurotransmitter (predominantly glutamate and neuropeptides) release from the nerve terminals (see 40 for review). Among excitatory neuropeptides present in vanilloid-sensitive nerves, substance P (SP) has been implicated in nociception. It has long been known that not only the release of SP is inhibited by vanilloid treatment, but SP is also depleted subsequently from primary sensory neurons of vanilloid-treated rats (75). SP is encoded by pre-protachykinin mRNAs (76), the transcription of which requires the presence of nerve growth factor (NGF) (77). NGF is produced in the periphery, from where it is transported intraxonally to the cell bodies of vanilloid-sensitive neurons (78). Since capsaicin treatment is known to block the intraaxonal transport of macromolecules by sensitive neurons (79), it is a reasonable assumption that a deprivation of NGF (and maybe also other intraaxonally transported neurotrophic factors) underlies the down-regulation of SP. Inflamed tissues produce increased amounts of NGF which is supposed to contribute to the development of inflammatory hyperalgesia (80). By blocking the transport of overproduced NGF, vanilloids may be beneficial in the treatment of such disease states.

Following vanilloid treatment the conductivity of C-fibers is reduced (81). Electrical stimulation of the sural nerve is still able to evoke the baseline flexor reflex (which is thought to be mediated by Aδ-fibers), but not the 'wind-up' phenomenon (equal trains of electrical stimuli evoke increasing reflex responses recorded from the hamstring muscles), which is linked to C-fibers (82). Furthermore, the spinal hyperexcitability, which normally follows the 'wind-up' in control animals, is diminished following RTX administration (82).

Vanilloids down-regulate the expression of their own receptors (see 39 for an overview). This vanilloid receptor loss takes more than six hours to develop, thus it is not rapid enough to be involved in acute desensitization. On the other hand, it is long lasting and in the urinary bladder the recovery of specific RTX binding sites parallel the restoration of the xylene-induced neurogenic plasma extravasation response. Therefore ligand-induced loss of vanilloid receptors may represent an important mechanism for long-term desensitization.

Whereas it has long been known that capsaicin down-regulates the expression of a number of neuropeptides, such as SP or calcitonin gene-related peptide (CGRP), normally present in vanilloid-sensitive neurons (reviewed in 40), it is a recent discovery that vanilloids at the same time can up-regulate other neuropeptides (for example, galanin, vasoactive intestinal polypeptide, and cholecystokinin), as well as the enzyme nitric oxide synthase (NOS) (reviewed in 83). These vanilloid-induced changes in neuropeptides expression by and large mimic those observed after mechanical nerve injury, collectively referred to as messenger plasticity (84). As galanin (presumably by binding to galanin receptors on second order dorsal horn neurons) is thought to exert inhibitory effect on spinal nociceptive input (85), increased synthesis of galanin may contribute to the prolonged analgesic action of vanilloids (82). Of relevance are the findings that the galanin receptor antagonist, M35, restored the reflex hyperexcitability (see above) in RTX-treated rats, and that an inverse correlation was observed between the number of dorsal root ganglia neurons positive for galanin mRNA and the responsiveness of the animals to noxious heat (82).

Capsaicin and Resiniferatoxin Actions: Similarities and Differences

RTX mimics most (but not all) capsaicin-like responses. However, as discussed above, RTX and capsaicin show striking differences in relative potencies to excite and desensitize different biological end-points. Generally speaking, RTX favors desensitization whereas capsaicin excels in excitation. A dramatic example of this phenomenon is the pulmonary chemoreflex (also known as the Bezold-Jarisch reflex) which is desensitized by RTX without prior excitation, but is evoked by capsaicin without subsequent desensitization (86). A number of mechanisms, which may underlie the differences between RTX and capsaicin actions, are discussed briefly below.

1) Putative vanilloid receptor subclasses may recognize RTX and capsaicin with diverse relative potencies. Of relevance may be the finding that whereas RTX is more than ten thousand-fold more potent than capsaicin for binding to cultured dorsal root ganglia neurons, it is only 300-fold more active than capsaicin for inducing calcium uptake under similar conditions (51).

2) RTX and capsaicin evoke inward ion currents with distinct kinetic properties (50). Whereas capsaicin-induced currents are rapid in onset and brief in duration, RTX-evoked currents, by contrast, tend to be slowly developing and prolonged. Consequently, capsaicin-induced currents are burst-like (activating voltage-dependent Na^+ channels), whilst RTX-induced currents are rather sustained (inhibiting voltage-dependent Na^+ channels). Nevertheless, the sustained RTX-induced currents deliver calcium in quantities sufficient to desensitize the nerves. Using

this model, it is easy to visualize why RTX is much more powerful to desensitize than to excite certain end-points.

3) Since vanilloid-sensitive neurons are heterogenous both in their size and their neurochemical properties, it is not unlikely that they are also diverse functionally. If so, it is important to note that a subset of primary sensory neurons respond exclusively to capsaicin (50). Probing further the overlap between neurons excited by RTX and capsaicin, on the one hand, and more selective vanilloids (for example, phorboid 20-homovanillates, devoid of hypothermia), on the other hand, might help identify functionally distinct subsets of vanilloid-sensitive neurons.

4) Since RTX is a bulkier and more lipophilic molecule than capsaicin, its tissue penetration rate is presumably much slower. As follows, capsaicin is likely to penetrate to the receptors rapidly and open the receptor-linked channels almost simultaneously whereas RTX probably occupies vanilloid receptors in a gradual manner. Such pharmacokinetic differences may predominate upon topical application, providing a rational explanation why RTX given intraocularly has an unexpectedly poor potency to provoke the chemogenic pain response (37).

5) Since the well-known drug-binding plasma protein, α_1-acid glycoprotein (AGP; also known as orosomucoid), binds vanilloids *in vitro* (87), it is safe to assume that vanilloids are transported in the blood in an AGP-bound form. Based on the concentration of AGP in the plasma and the affinities of AGP for capsaicin and RTX, respectively, it was estimated that more than 70% of capsaicin remains free (unbound) in the plasma as opposed to the much lower (13%) percentage free value for RTX (87). It is clear that the low plasma binding of capsaicin may lead to a rapid, hit-like response, which dissipates as capsaicin is eliminated from the plasma. By contrast, the high plasma binding of RTX may provide the biochemical basis for a prolonged and sustained biological action.

Concluding Remarks

The medicinal use of Euphorbium, the dried latex of *E. resinifera* Berg, reflects a history more than 2000 years old, which makes resiniferatoxin (RTX) one of the most ancient drugs still in use today. Some uses of Euphorbium, such as its application on nerves to suppress chronic pain or on dental cavities to mitigate tooth ache, give credit to doctors of bygone ages; others are rather puzzling or weird. With the benefit of hindsight, it must have been difficult to use Euphorbium in proper dosage as its RTX content varies substantially. No wonder that Euphorbium had become obsolete in medical practice by the turn of our century. RTX, the active principle in Euphorbium, was isolated only two decades ago, but it was not until 1989 that RTX was identified as an ultrapotent analog of capsaicin, the pungent ingredient in hot peppers. Based on their shared chemical trait, RTX and capsaicin structural analogs are collectively referred to as vanilloids. Specific binding of [^{3}H]RTX provided the first direct proof for the existence of a vanilloid (capsaicin) receptor. There is accumulating evidence to suggest the existence of a vanilloid receptor family, with more complex pharmacology than anticipated. Capsaicin has been the focus of attention as a potential non-steroid, non-narcotic analgesic and anti-inflammatory drug for decades, but the clinical use of capsaicin is severely limited by its irritancy and its relatively low efficacy. Worse yet, during the hepatic metabolism of capsaicin, toxic intermediates may be formed, making capsaicin a 'double-edged sword'. RTX, being an ultrapotent but relatively less pungent capsaicin analog, promises to be a better remedy. Animal experimentation suggets that, unlike capsaicin, RTX has a wide therapeutic window: a full desensitization against thermal or chemogenic pain can be achieved by means of a single RTX injection, without causing in the animals any signs of apparent toxicity. At present, RTX is undergoing clinical trials in patients suffering from painful

diabetic neuropathy. Nevertheless, it remains to be seen whether RTX itself lives up to the expectations as an analgesic drug.

Acknowledgments

We thank Drs. Peter M. Blumberg (National Cancer Institute, Bethesda, MD) and James E. Krause (Washington University School of Medicine, St. Louis, MO) for reading this manuscript.

References

1 A. TSCHIRCH and D. STOCK, *Die Harze*, 1635-1670, Borntraeger Verlag, Berlin (1935).
2. L. TURNER. *Euphorbias*, Batsford Ltd, London (1995).
3. A. BENEDICENTI, *Malati, Medici e Farmacisti*, 185-190, Hoepli, Milano (1947).
4. H. HULLEMAN, *De Vita et Scriptis Jubae*, Utrecht (1845).
5. A. TSCHIRCH, *Handbuch der Pharmacognosie*, Vol. III, 1167-1172, Tauchnitz Verlag, Leipzig (1923).
6. A. GOERLITZ, *Jubae II Regis Mauritaniae, Vita et Fragmenta*, Vol. I, Breslaw (1849); Vol. II, Breslaw (1862).
7. GAIUS PLINIUS SECUNDUS, *Naturalis Historia*, V. 16; X, 1; XXV, 38; XXV, 78.
8. P. SANGIORGIO, *Istoria delle piante medicate*, 588, Milano (1809).
9. SCRIBONIUS LARGUS, *Compositiones Medicamentorum*, circa 1th century A.D.
10. QUINTUS SERENUS (SAMMONICUS), *Liber medicinalis*, LV, 1000-1001.
11. DIOSCORIDES, *De Materia Medica*, III, 86.
12. S.B. CHRISTENSEN and K. SCHAUMBURG, J. Org. Chem. **48** 396 (1983).
13. Leide Rijksuniversiteitsbibliothek, ar 289, f 132 recto (dated 1083).
14. Bibliotheca Universitaria di Bologna, ar 1954, f 160 recto (dated 1244).
15. P. A. MATTHIOLUS, *Pedacio Dioscorides*, XC, 538 (1544).
16. *Farmacopea Italiana*, Vol. II, 1061, Torino.
17. *Pharmacopée Universelle*, 602, Baillière, Paris (1840).
18. M. GEOFFROY, *Traité de Matière Médicale*, Vol. IV, 210, Paris (1743).
19. *Merck's Index*, 3rd Edition, Merck & Co, Rahway, NJ (1920).
20. *Mercks's Index*, 4th Edition, Merck & Co, Rahway, NJ (1930).
21. P. MELIUS, *Herbarium*, Heltai Gáspárnè mühelye (in Hungarian), Kolozsvár (1578).
22. *Medicamenta*, VI Edizione, Vol. IV, 3447, Cooperativa Farmaceutica, Milano (1965).
23. F.V. MÉRAT and A.J. DE LENS, *Dictionnaire Universel de Matière Médicale*, 184-186, Ballière, Paris (1834).
24. H. JACKSON, *Account of the empire of Morocco and the district of Suse*, 81, London (1809).
25. F.A. FLUECKIGER and D. HANBURY, *Histoire des drogues d'origine végétale*, 304, Odoin, Paris (1878).
26. O.C. BERG and H. SCHMIDT, Offizinelle Gewächse **IV** 24 (1863).
27. E. HECKER and S. SOSATH, CLB Chem. Lab. Betr. **1** 42 (1991).
28. M. HERGENHAHN, W. ADOLPH and E. HECKER, Tetrahedron Lett. **19** 1595-1598 (1975).
29. M. HERGENHAHN, PhD Thesis, University of Heidelberg (1974).
30. S. ZAYED, B. SORG and E. HECKER, Planta Med. 65-69 (1984).
31. P.M. BLUMBERG, Cancer Res. **48** 1-8 (1988).
32. K.R. GUSTAFSON, J.H. CARDELLINA II, J.B. MCMAHON, R.J. GULAKOWSKI, J. ISHITOYA, Z. SZALLASI, N.E. LEWIN, P.M. BLUMBERG, O.S. WEISLOW, J.A. BEUTLER, R.W. BUCKHIET JR, G.C. CRAGG, P.A. COX, J.P. BADER and M.R. BOYD, J. Med. Chem. **35** 1978-1986 (1992).
33. P.M. BLUMBERG, A. SZALLASI and G. ACS, *Capsaicin in the Study of Pain*, J.N. Wood (ed), 45-60, Academic Press, New York (1993).
34. P.E. DRIEDGER and P.M. BLUMBERG, Cancer Res. **40** 1400-1404 (1980).
35. P.E. DRIEDGER and P.M. BLUMBERG, Proc. Natl. Acad. Sci. USA **77** 567-571 (1980).

36. H. ZUR HAUSEN, G.W. BORNKAMM, R.J. SCHMIDT and E. HECKER, Proc. Natl. Acad. Sci. USA **76** 782-785 (1979).
37. A. SZALLASI and P.M. BLUMBERG, Neuroscience **30** 515-520 (1989).
38. A. SZALLASI, Gen. Pharmacol. **25** 223-243 (1994).
39. A. SZALLASI, Acta Physiol. Scand. **155** (suppl. 629) 1-68 (1995).
40. P. HOLZER, Pharmacol. Rev. **43** 144-201 (1991).
41. N. JANCSÓ, *Pharmacology of Pain*, K. Lin, D. Armstrong and E.G. Pardo (eds), 33-55, Pergamon Press, Oxford (1968).
42. G. JANCSÓ, E. KIRÁLY and A. JANCSÓ-GÁBOR, Nature **270** 741-743 (1978).
43. A. SZALLASI and P.M. BLUMBERG, Life Sci. **47** 1399-1408 (1990).
44. C.A. MAGGI, R. PATACCHINI, M. TRAMONTANA, R. AMANN, S. GIULIANI and P. SANTICIOLOI, Neuroscience **37** 531-539 (1990).
45. A. SZALLASI, M. JONASSOHN, G. ÁCS, T. BÍRÓ, P. ÁCS, P.M. BLUMBERG and O. STERNER, Br. J. Pharmacol., in press.
46. A. SZALLASI and P.M. BLUMBERG, Brain Res. **524** 106-111 (1990).
47. S. BEVAN, S. HOTHI, G. HUGHES, I.F. JAMES, H.P. RANG, K. SHAH, C.S.J. WALPOLE and J.C. YEATS, Br. J. Pharmacol. **107** 544-552 (1992).
48. G. ACS, M. PALKOVITS and P.M. BLUMBERG, Life Sci. **55** 1017-1026 (1994).
49. A. SZALLASI, S. NILSSON, T. FARKAS-SZALLASI, P.M. BLUMBERG, T. HÖKFELT and J.M. LUNDBERG, Brain Res. **703** 175-183 (1995).
50. L. LIU and S.A. SIMON, J. Neurophysiol. **75** 1503-1514 (1996).
51. G. ACS, J. LEE, V.E. MARQUEZ and P.M. BLUMBERG, Mol. Brain Res. **35** 173-182 (1996).
52. C.D. GRIFFITHS, T.P.D. ELDERSHAW, D.P. GERATHY, J.L. HALL and E.Q. COLQUHOUN, Life Sci. **59** 105-117 (1996).
53. A. DICKINSON, C. HUGHES, A. RUEFF and A. DRAY, Pain **43** 353-362 (1990).
54. G. APPENDINO, G. CRAVOTTO, G. PALMISANO, R. ANNUNZIATA and A. SZALLASI. J. Med. Chem. **39** 3123-3131 (1996).
55. S. BEVAN and J. SZOLCSÁNYI, Trends Pharmacol. Sci. **11** 330-333 (1990).
56. S.J. BEVAN and R.J. DOCHERTY, *Capsaicin in the Study of Pain*, J.N. Wood (ed), 27-44, Academic Press, New York (1993).
57. J.C.G. COLERIDGE and H.M. COLERIDGE, Rev. Physiol. Biochem. Pharmacol. **99** 1-110 (1984).
58. A. JANCSÓ-GÁBOR, J. SZOLCSÁNYI and N. JANCSÓ, J. Physiol. (London) **206** 495-507 (1970).
59. M. SZIKSZAY and E.D. LONDON, Neuroscience **25** 917-923 (1988).
60. S. RITTER and T.T. DINH, *Capsaicin in the Study of Pain*, J.N. Wood (ed), 105-138, Academic Press, New York (1993).
61. A. SZALLASI and P.M. BLUMBERG, Life Sci. **48** 1863-1869 (1991).
62. U. OH, S.W. HWANG and D. KIM, J. Neurosci. **16** 1659-1667 (1996).
63. K. MADERSPACH and CS. FAJSZI, Biochim. Biophys. Acta **692** 469-478 (1982).
64. A. SZALLASI, G. ACS, G. CRAVOTTO, P.M. BLUMBERG, J.M. LUNDBERG and G. APPENDINO, Eur. J. Pharmacol. **299** 221-228 (1996).
65. A. SZALLASI, P.M. BLUMBERG and J.M. LUNDBERG, Eur. J. Pharmacol. Mol. Pharmacol. Section **289** 181-187 (1995).
66. S. BEVAN and G. GEPPETTI, Trends Neurosci. **17** 509-512 (1994).
67. G. ACS, M. PALKOVITS and P.M. BLUMBERG, J. Pharmacol. Exp. Ther. **274** 1090-1098 (1995).
68. A. SZALLASI, P.M. BLUMBERG, T. HÖKFELT and J.M. LUNDBERG, Eur. J. Pharmacol. **298** 321-327 (1996).
69. R. AMANN and C.A. MAGGI, Life Sci. **49** 849-856 (1991).
70. R.J. DOCHERTY, B. ROBERTSON and S. BEVAN, Neuroscience **40** 513-521 (1991).
71. J.S. HARVEY, C. DAVIS, I.F. JAMES and G.M. BURGESS, J. Neurochem. **65** 1309-1317 (1995).
72. J.C. YEATS, H.W.G.M. BODDEKE and R.J. DOCHERTY, Br. J. Pharmacol. **107** (Suppl) 238P (1992).
73. G. JANCSÓ, E. KIRÁLY, A. SZEBENI, L. TÓTH, E. BÁCSY, F. JOÓ and Á. PÁRDUCZ, Brain Res. **295** 211-216 (1984).
74. J. SZOLCSÁNYI, *Capsaicin in the Study of Pain*, J.N. Wood (ed), 1-26, Academic Press, New York (1993).
75. T.M. JESSELL, L.L. IVERSEN and A.C. CUELLO, Brain Res. **152** 183-188 (1978).

76. J.E. KRAUSE, J.M. CHIRGWIN, M.S. CARTER, Z.S. XU and A.D. HERSHEY, Proc. Natl. Acad. Sci. USA **84** 881-885 (1987).
77. R.M. LINDSAY and A.J. HARMAR, Nature **337** 362-364 (1989).
78. R. LEVI-MONTALCINI and P.U. ANGELETTI, Physiol. Rev. **48** 534-569 (1968).
79. M.S. MILLER, S.H. BUCK, I.G. SIPES, H.I. YAMAMURA and T.F. BURKS, Brain Res. **250** 193-196 (1982).
80. G.L. LEWIN and L.M. MENDELL, Trends Neurosci. **16** 353-359 (1993).
81. R. BARANOWSKI, B. LYNN and A. PINI, Br. J. Pharmacol. **89** 267-276 (1986).
82. A. SZALLASI, X.-J. XU, T. FARKAS-SZALLASI, J.M. LUNDBERG, T. HÖKFELT and Z. WIESENFELD-HALLIN, *8th World Congress on Pain*, Vancouver, BC, Abstracts, 122, IASP Press, Seattle (1996).
83. A. SZALLASI, J. Peripheral Nerv. Syst. **1** 1-13 (1996).
84. T. HÖKFELT, X. ZHANG and Z. WIESENFELD-HALLIN, Trends Neurosci. **17** 22-30 (1994).
85. M. YANAGISAWA, N. YAGI, M. OTSUKA, C. YANAIHARA and N. YANAIHARA, Neurosci. Lett. **70** 278-282 (1986).
86. J. SZOLCSÁNYI, A. SZALLASI, Z. SZALLASI, F. JOÓ and P.M. BLUMBERG, J. Pharmacol. Exp. Ther. **255** 923-928 (1990).
87. A. SZALLASI, N.E. LEWIN and P.M. BLUMBERG, J. Pharmacol. Exp. Ther. **262** 883-888 (1992).