Neural Injury, Repair, and Adaptation in the GI Tract II. The elusive action of capsaicin on the vagus nerve

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Holzer, Peter. Neural Injury, Repair, and Adaptation in the GI Tract. II. The elusive action of capsaicin on the vagus nerve. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G8–G13, 1998.—Capsaicin is an excitotoxin for primary afferent neurons, and perivagal administration of capsaicin is frequently used to ablate afferent fibers from the vagus nerve in an attempt to elucidate the role of afferent fibers in gastrointestinal (GI) regulation. However, this method has recently been called into question by research demonstrating that the molecular target of capsaicin on spinal and trigeminal afferents, vanilloid receptor subtype 1 (VR1), is absent from vagal afferents. Although some concerns about selectivity exist, the available information suggests that perineural capsaicin defunctionalizes afferent fibers of the vagus nerve by acting on a vanilloid receptor subtype that is structurally different from VR1.

vanilloid receptors; vanilloid receptor heterogeneity; excitotoxicity; vagal afferents; spinal afferents

The vanilloid capsaicin is the pungent ingredient in red peppers of the genus Capsicum, including chilies and jalapeños. It was around 1950 that the Hungarian pharmacologist Nikolaus Jancsó realized that the sensation of burning pain elicited by capsaicin is caused by selective stimulation of nociceptive afferent neurons. In addition, Jancsó discovered that the vanilloid can induce a long-term blockade of those nociceptive afferents that are acutely stimulated by the drug (2, 9, 27). After a structure-activity relationship for the excitotoxic action of capsaicin had been disclosed, the hypothesis was put forward that capsaicin acts via specific cell membrane receptors (Fig. 1), which are exclusively expressed by nociceptive afferent neurons (2, 9, 27). The existence of vanilloid receptors has since been proven by the discovery of a specific receptor antagonist, capsazepine (27), by the demonstration of specific binding sites for the vanilloid-phorbol ester resiniferatoxin on dorsal root ganglion membranes (27, 28), and by the isolation and characterization of a functional cDNA encoding vanilloid receptor subtype 1 (VR1) (4).

CAPSAICIN IS AN EXCITOTOXIN ACTING VIA VR1

Structurally related to Ca\(^{2+}\) channels activated by stores of Ca\(^{2+}\), the rat VR1 is a polytopic protein that consists of 838 amino acids with a predicted molecular weight of 95,000, and contains six transmembrane domains (4). Functionally, VR1 is a nonselective cation channel that displays an exceptionally high permeability for Ca\(^{2+}\) and closely resembles the capsaicin-operated cation channel. The inward current carried by VR1 depolarizes the cells and in so doing elicits repetitive firing of action potentials (2, 4). In addition, the influx of Ca\(^{2+}\) causes exocytotic release of peptide neurotransmitters (Fig. 1), as has been observed at both the peripheral and central endings of primary afferent neurons (9). Interestingly, VR1 is activated not only by capsaicin and related vanilloids but also by an increase of the temperature into the noxious range, which indicates that it functions as a transducer of noxious heat (4). This intriguing aspect of VR1 is likely to explain the burning sensation of capsaicin-evoked pain.

If drug concentration and contact time exceed a certain limit, vanilloids exert an overt neurotoxic action (Fig. 1) that results in the death of dorsal root ganglion neurons in culture and long-term (several weeks to months) impairment of afferent neuron functions in vivo (2, 9, 27). This cytotoxic action is likewise mediated by VR1, since nonneuronal cells such as human embryonic kidney HEK293 cells transfected with VR1 are also killed if they are exposed to capsaicin (3 µM) for a prolonged period of time (4). The neurotoxic process initiated by capsaicin-evoked VR1 activation leads to necrotic cell death (4), which seems to result from two major mechanisms (2, 9, 27). One mechanism is related to excessive influx of Ca\(^{2+}\) into the cell, which leads to activation of Ca\(^{2+}\)-dependent enzymes and a long-lasting impairment of mitochondrial function (Fig. 1). The other mechanism involves influx of Na\(^{+}\) through VR1 and passive movement of Cl\(^{-}\) into the cell. The net uptake of NaCl is followed by an influx of water, swelling, and osmotic lysis of the cells (Fig. 1). Because the degeneration of afferent neurons varies with the nerve region and the age of the animal, it is obvious that the endpoint of the neurotoxic action of capsaicin in vivo is controlled by additional factors (9). In particular, there is a critical period in the ontogeny of unmyelinated afferents during which they are particularly vulnerable to capsaicin. Thus only a minor population of afferents is ablated when adult rats are treated with capsaicin, whereas a large percentage of dorsal root...
ganglion neurons degenerate after capsaicin administration in newborn rats (9, 11, 22).

**CAPSAICIN AS A PROBE FOR AFFERENT NEURON FUNCTIONS IN THE GUT**

The dorsal root ganglion cells, which are acutely excited and defunctionalized by capsaicin in the long term, can be roughly characterized as neurons that have small- to medium-sized cell bodies with mostly unmyelinated fibers (C fibers) and a few thinly myelinated fibers (Aδ fibers), which are particularly sensitive to chemicals and heat (9). It is obvious, therefore, that capsaicin-sensitive afferent neurons are heterogeneous and do not completely overlap with any group of afferents that has been classified according to morphological, neurochemical, or functional criteria (9). The usefulness of capsaicin as a neuropharmacological tool derives from the cellular selectivity of its action, since autonomic neurons, motoneurons, enteric neurons, and most central neurons are thought to be insensitive to vanilloids (2, 9, 11, 27). However, the selectivity of capsaicin for afferent neurons is not absolute; for instance, some neurons in discrete forebrain and hindbrain areas, including the preoptic area of the hypothalamus, are susceptible to the neurotoxic action of the drug (22), although they do not express vanilloid receptors (4, 28). Notwithstanding, capsaicin is widely used as a neuropharmacological tool to manipulate the activity of certain primary afferents and to explore their functional implications (9). Exploitation of the long-term neurotoxic action of capsaicin has gained particular popularity, because the capsaicin-induced knock-out of afferent neurons spares efferent autonomic neurons, which are unavoidably cut by nerve transection. The physiological roles of the neurons that have been defunctionalized by the neurotoxin are inferred from the functional deficits produced by capsaicin. Systemic treatment of experimental animals (mostly rats) with neurotoxic doses of capsaicin is the approach most frequently used; this method ablates all neurons sensitive and accessible to the drug. An alternative route of considerable potential is perineural application of the drug, which enables capsaicin to selectively ablate afferent fibers from mixed nerves that contain both afferent and efferent axons (9, 19, 20, 25).

Both approaches have been used to explore the functional implications of sensory neurons in the gastrointestinal tract. The gastrointestinal tract is supplied by two groups of extrinsic afferent nerve fibers (Fig. 2). The spinal afferents originate from the dorsal root ganglia and reach the gut via sympathetic (splanchnic, colonic, and hypogastric) and sacral parasympathetic (pelvic) nerves, whereas the vagal afferents emanate from the nodose (and jugular) ganglia and supply the GI tract down to the transverse colon. Consequently, perivagal application of neurotoxic concentrations of capsaicin is used to elucidate the implications of nodose ganglion neurons in the regulation of digestive functions (Table 1). Capsaicin administration has also been used to probe the function of spinal afferents passing through the celiac ganglion (19), although the celiac branch of the vagus nerve that supplies the intestine is also affected. A comparison of the effects of capsaicin given intraperitoneally, perivagally, intracerebroventricularly, or intrathecaly has revealed that the neurotoxic action of perivagal capsaicin is confined to the treated nerve (25). Systemic application of capsaicin is a convenient way to screen for the overall role of extrinsic afferent fibers in the regulation of digestive activity, although the results need to be interpreted cautiously in view of the toxic action of vanilloid on some central neurons (22), because any functional deficits in digestive activity may implicate both afferent and central neurons.

**THE ELUSIVE ACTION OF CAPSAICIN ON THE VAGUS NERVE IN THE ABSENCE OF VR1**

Studies involving perivagal administration of capsaicin imply that vagal afferents participate in many gastrointestinal functions that were previously thought to be independent of extrinsic afferents (Table 1). However, the relevance of these studies has been called into question by the recent finding that nodose ganglion neurons do not express VR1, the molecular target of the excitotoxic action of capsaicin on dorsal root and trigeminal ganglion neurons (4). The absence of VR1 from vagal afferents contrasts with the reported sensitivity
of these neurons to capsaicin and casts doubt on the selectivity of the action of capsaicin on the vagus nerve. This divergence and other inconsistencies in the effects of perivagal capsaicin call for a critical review of the validity of the contention that vagal afferents can be selectively stimulated and defunctionalized by capsaicin.

Although they do not express VR1, vagal afferents respond to capsaicin, and there is ample evidence to show that the excitotoxic effect of the drug on nodose ganglion neurons is similar to that on dorsal root ganglion neurons in terms of its potency and mechanism of action (9). Thus capsaicin depolarizes vagal afferents of the rat and guinea pig at concentrations of 0.03–3 µM, concentrations identical to those depolarizing spinal afferents, and causes disruption of the microtubular cell organization when rat nodose ganglion cells are exposed to drug concentrations of 1–10 µM for 5–10 min (6, 16, 30). The manifestations of the neurotoxic action of capsaicin on vagal afferents in vivo have been explored after systemic and perivagal administration of the vanilloid; these include functional deficits in the gastrointestinal tract (Table 1) and other vagally innervated organs such as the respiratory tract.

Systemic administration of capsaicin (50 mg/kg) to newborn rats reduces the number of small-diameter neurons in the nodose ganglia by up to 70% (3), which matches the extent of degeneration seen in the dorsal root ganglia (9, 11). Systemic treatment of adult rats with neurotoxic doses of capsaicin also causes some nodose ganglion neurons to degenerate (25), which is accompanied by a long-lasting depletion of substance P from the ganglion and vagus nerve (9). In the esophagus and stomach, about 90% of the intraganglionic and 60% of the intramuscular terminals of anterogradely labeled nodose ganglion neurons persist after systemic administration of capsaicin to adult rats, whereas in the small and large intestine virtually no vagal nerve endings survive (1). It follows that vagal afferents terminating in the muscle and myenteric plexus of the esophagus and stomach are mostly capsaicin resistant, whereas those innervating the small and large intestine are capsaicin sensitive (1).

Table 1. Gastrointestinal functions that are inhibited by perivagal administration of capsaicin in the rat

<table>
<thead>
<tr>
<th>Region</th>
<th>Function</th>
<th>References</th>
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<tbody>
<tr>
<td>Stomach</td>
<td>Increase in mucosal blood flow evoked by intracisternal TRH</td>
<td>18</td>
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<tr>
<td></td>
<td>Acid secretion evoked by intracisternal TRH</td>
<td>18</td>
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<tr>
<td></td>
<td>Acid secretion evoked by vagal nerve stimulation</td>
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<td></td>
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<tr>
<td></td>
<td>Inhibition of motility by duodenal distension</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Secretin-induced inhibition of motility</td>
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</tr>
<tr>
<td></td>
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<td>7, 25</td>
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<td></td>
<td>CCK-induced inhibition of motility and emptying</td>
<td>7, 20</td>
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<td>Small intestine</td>
<td>CCK-induced disruption of migrating motor complexes</td>
<td>23</td>
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<td></td>
<td>Disruption of myoelectric activity by anaphylaxis</td>
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<tr>
<td>Pancreas</td>
<td>CCK-induced exocrine secretion</td>
<td>12, 13</td>
</tr>
<tr>
<td></td>
<td>Exocrine secretion induced by intraduodenal cascin, maltose, or hypertonic saline</td>
<td>12, 13</td>
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TRH, thyrotropin-releasing hormone.
available information suggests that substantial numbers of vagal afferents are damaged or undergo degeneration, given that the retrograde transport of True blue injected into the gastric wall back to the nodose ganglion is prevented (12).

**TENTATIVE IMPLICATIONS OF CAPSAICIN-SENSITIVE VAGAL AFFERENTS IN GASTROINTESTINAL REGULATION**

Table 1 summarizes a number of studies in which application of a neurotoxic concentration of capsaicin to rat vagus nerves was found to inhibit various digestive functions. Some studies have compared the effects of systemic, perivagal, and periceliac administration of the excitotoxin in a systematic attempt to analyze whether vagal or spinal afferents, or both, contribute to the function under study. From the available information it would appear that capsaicin-sensitive fibers running in the vagus nerves contribute to a number of motor, secretory, and circulatory processes in the upper gut (Table 1). Other digestive activities seem to involve both vagal and spinal afferents, because perivagal as well as periceliac application of capsaicin are both able to attenuate the function under study. This situation applies to the inhibitory actions of CCK, duodenal acidification, and duodenal distension on gastric motor activity and emptying (7, 8, 17, 20). In contrast, the rise of gastric blood flow that ensues in response to acid back-diffusion and the shutdown of gastrointestinal motility after abdominal surgery seem to be exclusively brought about by spinal afferents (10, 19).

Some studies involving perivagal and other routes of capsaicin application have apparent discrepancies in their results. For instance, gastric acid secretion evoked by vagal nerve stimulation has been found to be either unchanged or attenuated by perivagal capsaicin, whereas systemic capsaicin is ineffective (10, 18, 24). Another apparent inconsistency of physiological significance relates to the role vagal afferents play in the action of exogenous and endogenous CCK on pancreatic exocrine secretion. Li et al. (12) and Li and Owyang (13) contend that physiological plasma concentrations of CCK stimulate pancreatic enzyme secretion via a reflex that involves capsaicin-sensitive vagal afferents; however, the studies of Guan et al. (7) negate this mechanism, because in their experiments CCK-evoked secretion of pancreatic enzymes remained unaltered by perivagal administration of capsaicin.

**CONCERNS ABOUT THE SELECTIVITY OF PERIVAGAL CAPSAICIN FOR AFFERENT NERVE FIBERS**

Because the molecular target of capsaicin on dorsal root and trigeminal ganglion neurons, VR1, is either absent from nodose ganglion cells or expressed at an undetectably low level (4), the possibility exists that the functional deficits caused by perivagal capsaicin reflect a nonspecific action of the vanillloid on afferent and/or efferent neurons of the vagus nerve. Given that 80–90% of the fibers in the vagus nerve are afferent, it appears plausible that damage to the quantitatively minor population of efferent neurons could to some extent be overlooked. Moreover, the perineural treatment protocol involves the use of a very high concentration of 1% (33 mM) capsaicin, which raises possible concerns about the selectivity with which perivagal capsaicin acts on vagal afferents, since low micromolar concentrations of the drug are known to inhibit many excitable cells other than primary afferent neurons (2, 9, 27). These cell-nonspecific effects of capsaicin, which include inhibition of gastrointestinal smooth muscle activity, are not related to VR1-mediated excitotoxicity and arise from inhibition of voltage-gated Na+ and K+ channels (2, 9, 27). Although the cell-nonspecific effects of capsaicin abate relatively quickly in comparison with the long-term consequences of primary afferent neurotoxicity (9), it cannot be ruled out that some of the nonspecific actions persist, as is true for the capsaicin-induced degenerative changes of central neurons (22, 25), which do not express vanillloid receptors (4, 28). Problems may also arise from the toxic vehicle additives Tween 80 and ethanol, which are used to solubilize capsaicin. Ethanol (10%) is known to cause a temporary blockade of nerve conduction not only in afferent but also in efferent fibers of the vagus nerve (26), whereas the toxic profile of Tween 80 (10%) on vagal nerve integrity remains unexplored.

These concerns are alleviated by some studies that have proven the selectivity of perivagal capsaicin in ablating afferent neurons by demonstrating that responses to stimulation of vagal efferents remain intact. Thus gastric motor activity evoked by vagal nerve stimulation (15, 20) or intracisternal injection of thyrotropin-releasing hormone (18) and pancreatic enzyme secretion elicited by 2-deoxy-D-glucose, which is known to stimulate vagal efferents (7, 13), remain unchanged by perivagal administration of capsaicin. In contrast, the significance of the finding that gastric acid secretion in response to vagal nerve stimulation is attenuated by perivagal capsaicin (24) is not fully understood. Hence we cannot dismiss the possibility that different degrees of damage to vagal efferents account for the discrepant effects of perivagal capsaicin on gastric acid secretion due to vagal nerve stimulation (9, 24) and on pancreatic exocrine secretion due to CCK (7, 12, 13). Thus, although there is functional evidence that afferent neurons are ablated by perivagal administration of capsaicin (12, 15), there is little neuroanatomic proof that ablation is confined to afferents and does not involve some efferents as well. Consequently there is a need to precisely determine the selectivity with which perineural capsaicin acts on vagal afferents and efferents in terms of morphological and functional integrity and to determine ways in which nonselective effects of capsaicin and its vehicle can be minimized.

**SELECTIVE ACTION OF CAPSAICIN ON VAGAL AFFERENTS VIA A RECEPTOR THAT IS DISTINCT FROM VR1**

Despite concerns about the cellular selectivity of perivagal capsaicin, it is important to emphasize that the excitotoxic action of capsaicin on vagal afferents
resembles that on spinal afferents so closely (6, 16, 30) that a common molecular mechanism of action can be envisaged. Indeed, biochemical and autoradiographic studies have shown that specific vanilloid receptor binding sites labeled by [3H]resiniferatoxin, an ultrapotent analog of capsaicin, are expressed not only by spinal and trigeminal afferents but also by vagal afferents (27, 28). These vanilloid binding sites are present in the nodose ganglion and transported in the vagus nerve in an anterograde direction but also occur in the nucleus of the solitary tract and area postrema, the central projection areas of vagal afferents (28). Although the specific vanilloid binding sites on nodose ganglion neurons do not differ from those on dorsal root ganglion neurons in terms of affinity, positive cooperativity, and density (27, 28), they cannot be identical to VR1, which is expressed by dorsal root but not nodose ganglion neurons (4). There are compelling reasons, therefore, to infer that vagal afferent neurons express vanilloid receptors that have a similar affinity to capsaicin—VR1 as VR1 but a molecular structure distinct from that of VR1. This conjecture fits with other lines of evidence for vanilloid receptor heterogeneity (27).

Expression of a separate subtype of vanilloid receptor is one of many neurochemical and functional traits that differentiate nodose ganglion neurons from dorsal root ganglion neurons (Fig. 2). Importantly, the spinal VR1 and the vagal vanilloid receptor differ not only in their cellular localization and molecular structure but also in their regulation, given that the long-term expression of capsaicin sensitivity in cultured dorsal root ganglia requires the presence of nerve growth factor, whereas that of nodose ganglia depends on brain-derived neurotrophic factor (30). This differential requirement of regulatory factors is consistent with the dependency of vagal afferents on brain-derived neurotrophic factor and neurotrophin-3 for their development and maintenance, whereas spinal afferents require nerve growth factor for that purpose. Together with their different embryonic origin, vagal and spinal afferents thus represent two largely divergent groups of extrinsic sensory neurons supplying the gut (Fig. 2). Once the vanilloid receptor subtype expressed by nodose ganglion neurons has been characterized in its molecular structure, it should become possible to design ligands that are selective for this receptor subtype and hence could be used to selectively target vagal afferents.

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