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Review

Functional and chemical anatomy of the afferent vagal system

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Abstract

The results of neural tracing studies suggest that vagal afferent fibers in cervical and thoracic branches innervate the esophagus, lower airways, heart, aorta, and possibly the thymus, and via abdominal branches the entire gastrointestinal tract, liver, portal vein, billiary system, pancreas, but not the spleen. In addition, vagal afferents innervate numerous thoracic and abdominal paraganglia associated with the vagus nerves. Specific terminal structures such as flower basket terminals, intraganglionic laminar endings and intramuscular arrays have been identified in the various organs and organ compartments, suggesting functional specializations. Electrophysiological recording studies have identified mechano- and chemo-receptors, as well as temperature- and osmo-sensors. In the rat and several other species, mostly polymodal units, while in the cat more specialized units have been reported. Few details of the peripheral transduction cascades and the transmitters for signal propagation in the CNS are known. Glutamate and its various receptors are likely to play an important role at the level of primary afferent signaling to the solitary nucleus. The vagal afferent system is thus in an excellent position to detect immune-related events in the periphery and generate appropriate autonomic, endocrine, and behavioral responses via central reflex pathways. There is also good evidence for a role of vagal afferents in nociception, as manifested by affective-emotional responses such as increased blood pressure and tachycardia, typically associated with the perception of pain, and mediated via central reflex pathways involving the amygdala and other parts of the limbic system. The massive central projections are likely to be responsible for the antiepileptic properties of afferent vagal stimulation in humans. Furthermore, these functions are in line with a general defensive character ascribed to the vagal afferent, paraventricular system in lower vertebrates. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Vagus nerve; Visceral afferents; Paraganglia; Nodose ganglion; Nucleus of the solitary tract; Immunosensing; Nociception; Defensive mechanisms

1. Introduction

The vagus nerve is different from the other cranial nerves in that it predominantly supplies the thoracic and abdominal cavities. It got the name from the ‘wandering’ character of its complex course along the esophagus and major arteries. Considering the theme of the present volume, the goal of this brief review is to provide a sound background of the anatomical aspects of this ‘wanderer’ and relate them to specific functions as they are known, in particular to its potential role in thermoregulation and immune-brain communication. Clearly the nerve itself is merely a cable, and the most interesting parts are its peripheral interface with specific sensors and effectors, as well as its central interface with the rest of the brain. Nevertheless, knowledge of the branching pattern of the nerve and its variations in individual animals is important for selective vagotomy experiments. In addition, the nerve trunks themselves contain more than just axons and glial cells. The presence of dendritic cells belonging to the immune defense system has been recently demonstrated in cervical and subdiaphragmatic vagal trunks (Goehler et al., 1999). In addition, paraganglia are often embedded in the major trunks near branch points (Prechtl and Powley, 1985; Kummer and Neuhuber, 1989; Dahlqvist et al., 1994). Therefore, we will first have a look at the gross anatomy and branching pattern of the vagus nerves and then focus on the peripheral and central interfaces.

2. Gross anatomy

2.1. Cervical and thoracic vagus

The left and right cervical vagi are formed by converging afferent and efferent rootlets as they leave the cranium through the jugular foramen (Fig. 1). Immediately outside the cranium lie the proximal jugular and the slightly more

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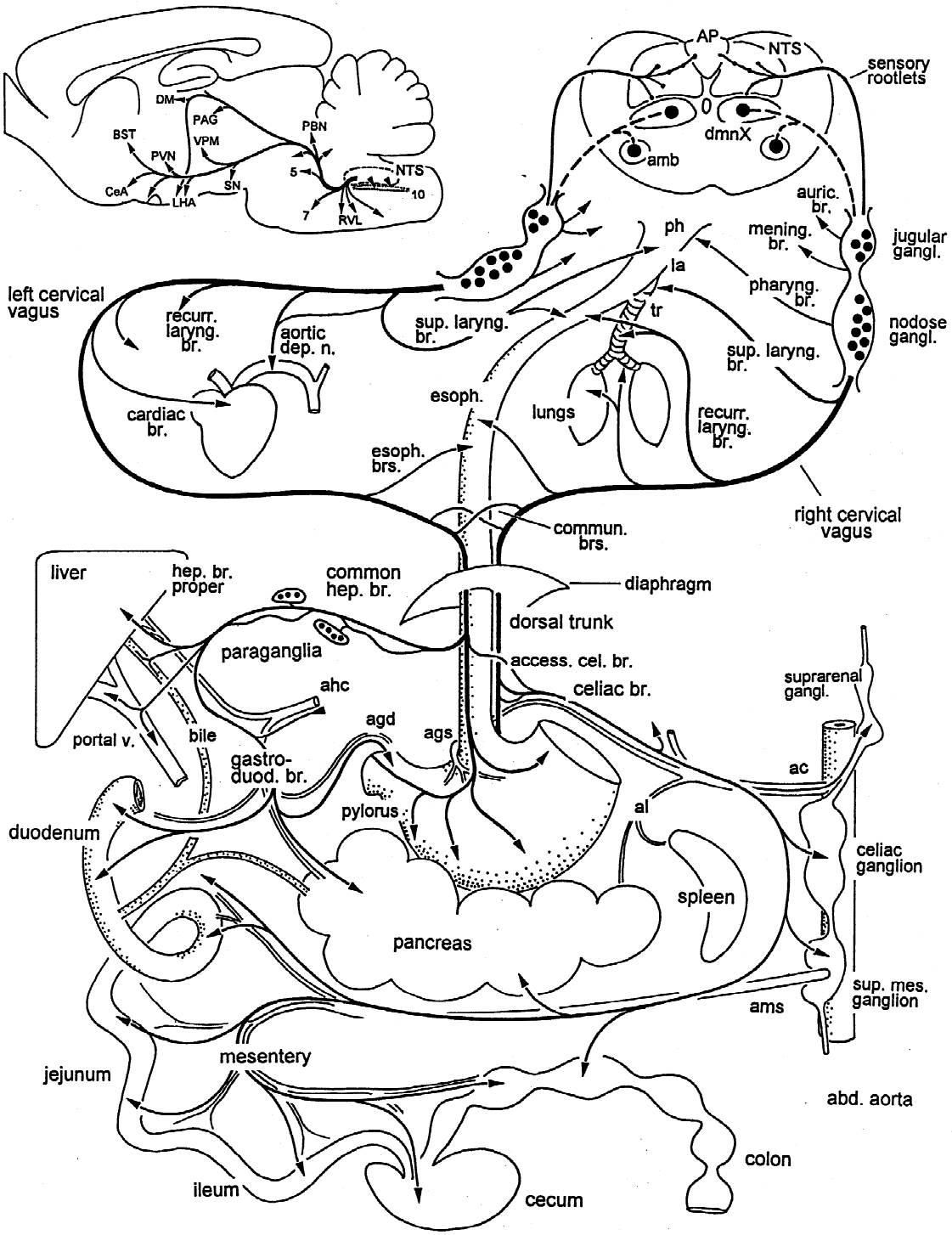


Fig. 1. Schematic representation of the gross anatomical distribution of the vagus nerve, and central distribution of vagal afferent information from the NTS. Some peripheral vagal branches may not carry afferent and efferent fibers. All cervical and thoracic branches are bilateral and may have been omitted for clarity. Abbreviations for periphery: ac, celiac artery; agd, right gastric artery; ags, left gastric artery; ahc, common hepatic artery; al, splenic artery; ams, superior mesenteric artery; la, larynx; ph, pharynx; tr, trachea. Abbreviations for brain areas: Amb, nucleus ambiguus; AP, area postrema; BST, bed nucleus of stria terminalis; DM, dorsomedial nucleus of thalamus; CeA, central nucleus of amygdala; LHA, lateral hypothalamic area; NTS, nucleus tractus solitarius; PAG, periaqueductal gray; PVN, paraventricular nucleus of hypothalamus; PBN, parabrachial nucleus; RVL, rostroventrolateral medulla; SN, substantia nigra; VPM, ventral posteriomedial nucleus of thalamus; 5, trigeminal nucleus; 7, facial nucleus; 10 (dmnX), dorsal motor nucleus of the vagus.

distal nodose ganglia, which harbor the neuronal cell bodies of the sensory neurons. The cervical vagus nerve runs parallel to the carotid artery on its lateral side. The first branches leaving the cervical vagus at the level of the jugular ganglion are the auricular and meningeal branches, providing sensory innervation to the skin of the external

acoustic meatus and the dura of the posterior cranial fossa, respectively. The pharyngeal branch (or several branches in larger species) splits off at the level of the nodose ganglion. At its caudal pole branches off the superior laryngeal nerve, coursing beneath the carotid artery to the larynx but also providing small branches to the caudal pharynx and upper esophagus. A cervical cardiac branch (or several branches in larger species) is given off along the course of the cervical vagus or in some cases from the superior laryngeal nerve. This nerve branch is also termed aortic branch (Helke et al., 1980) or depressor nerve and contains mainly afferent fibers from barosensors of the aortic arch. The recurrent laryngeal nerve leaves the vagus nerve at the level of the subclavian artery on the right side, and the aortic arch on the left side. Looping around these two blood vessels, it continues cranially, closely attached to the trachea and esophagus which it innervates. In the upper mediastinum, branches are given off to the main bronchi and lungs, and to the heart. Further caudally, both vagal nerves accompany the esophagus forming a more or less well developed plexus in its adventitia. Passing together with the esophagus through the diaphragm, the left and right vagus become contiguous with the anterior and posterior vagal trunks, respectively.

Thoracic and abdominal communicating branches between the left and right trunks have been described in some species, with afferent and/or efferent axons from one side crossing over to the other side (Agostoni et al., 1957; Prechtl and Powley, 1985; Fitzakerley and Lucier, 1988). In the rat, there is little evidence from retrograde tracing experiments for crossing efferent axons, but as many as 20% of afferent axons may cross at the thoracic level (Sauter et al., 1983). In the ferret an abdominal communicating branch has been characterized, with both efferent and afferent axons crossing sides (Fitzakerley and Lucier, 1988).

2.2. Abdominal vagus

The branching pattern in the abdomen has been best characterized in the rat (Prechtl and Powley, 1985), and is generally similar in most other species studied, including man. The ventral or anterior trunk, which is contiguous with the left cervical vagus, branches into the common hepatic, ventral gastric and ventral (or accessory) celiac branches (Fig. 1). The common hepatic branch divides typically at about one third the distance between diaphragm and gastric cardia, but this is variable (Prechtl and Powley, 1985). Furthermore, double dorsal trunks have been observed in the rat (Prechtl and Powley, 1985) and other species. Using electron microscopy on cross sectioned nerves, and supra- vs. infranodose cervical, thoracic, or abdominal vagotomies, the proportion of myelinated and unmyelinated axons as well as afferent, efferent and adventitial axons have been estimated in the rat (Prechtl and Powley, 1990), cat (Agostoni et al., 1957), rabbit (Evans and Murray, 1954) and ferret (Asala and Bower, 1986). In the rat there are about 11,000 nerve fibers in each of the subdiaphragmatic trunks, of which about 8000 are afferent and 3000 efferent. Less than 1% of all fibers are myelinated, and the average size of the unmyelinated fibers is about 0.8 μm, with some axons as thin as 0.1 μm.

The common hepatic branch of the abdominal vagus has received the most attention with regard to immune-brain communication and thermoregulation. The common hepatic branch contains a total of about 3000 fibers; 2200 of which are afferent, 200 efferent, and 600 are adventitial, non-vagal fibers (Prechtl and Powley, 1990). This branch is usually referred to as simply ‘hepatic’ branch, but this is clearly a misnomer, because the majority of its axons do not supply the liver, but instead the pylorus, antrum, pancreas and proximal duodenum (Berthoud et al., 1992; Phillips et al., 1997). As the common hepatic branch reaches the hepatic artery proper (via the hepato-esophageal artery), it runs towards the common hepatic artery and then with the gastroduodenal artery, to ultimately divide into branches following the right gastric artery to the gastric antrum and pyloric sphincter and the ventral and dorsal duodenopancreatic arteries to the proximal duodenum and head of the pancreas (Fig. 1). Therefore, the hepatic branch should be named common hepatic branch dividing into the hepatic branch proper and the gastroduodenal or pyloric branches (Berthoud et al., 1992). This has important implications for the functional interpretation of experiments in which the common hepatic branch is cut (see below).

The ventral or anterior gastric branch supplies the ventral side of the stomach and through small fascicles within the circular muscle of the pyloric sphincter it also innervates the proximal duodenum. The dorsal or posterior gastric branch enters the dorsal side of the stomach near the cardia along the left gastric artery. It too supplies the proximal duodenum via transpyloric fibers. The ventral celiac branch, after wrapping around the ventral esophagus, joins the dorsal celiac branch near the origin of the left gastric artery. Both branches descend on the celiac artery to near the celiac ganglion, and then distribute to the small and large intestines along the superior mesenteric artery and its mesenteric offshoots.

3. What structures are innervated by vagal afferents?

3.1. Methodological considerations

With the advent of neuronal tracing, the earliest studies used tritiated amino acids injected into the nodose ganglia (Sato and Koyano, 1987). Later, vagal afferents were labeled with injections of DiI, an intensely orange fluorescent carbocyanine dye (1,1′-dioleyl-3,3,3′,3′-tetramethylindo-carbocyanine; Berthoud and Powley, 1992) or

wheat germ agglutinin-horseradish peroxidase (WGA-HRP; Neuhuber, 1987) into the nodose ganglia. Unlike the sympathetic postganglionics and dorsal root afferents where tyrosine hydroxylase and calcitonin gene-related peptide are excellent neurochemical markers, there are no such unifying markers for vagal afferents (or efferents) that could be exploited for immunohistochemistry. An exception might be calretinin, which is a quite selective marker for at least one population of vagal afferents innervating the proximal esophagus (Dütsch et al., 1998).

A comprehensive account of visceral structures innervated by vagal afferents was thus only possible with neuronal tracing. The fluorescent tracer DiI and WGA-HRP have been used successfully to label vagal afferents in the rat and mouse in vivo. In the case of DiI, vagal afferents and terminals are intensely labeled throughout the innervated thoracic and abdominal organs, 2–6 weeks after injection into the nodose ganglia. Because of the strong fluorescence and no requirement for histological processing, the method lends itself ideally for whole-mounting and confocal microscopy (Berthoud and Powley, 1992; Berthoud et al., 1992, 1995a,b, 1997a). If none, or weak detergents are used, DiI-labeling can be combined with immunohistochemistry for neuronal or other cellular markers (Williams et al., 1997). WGA-HRP has the advantage that it is water soluble, and requires only short transport times of 1–3 days (Neuhuber, 1987; Phillips et al., 1997). Labeled material can be processed with DAB (Neuhuber, 1987), tetramethyl benzidine (Kalia and Mesulam, 1980a,b; Neuhuber, 1987; Phillips et al., 1997), or with a recently described method using fluorescently labeled streptavidin (Kressel, 1998). This latter method allows easy combination with fluorescence immunohistochemistry for one or more antigens with simultaneous or sequential processing (Kressel and Radespiel-Tröger, 1999; Berthoud and Patterson, 2000).

3.2. Lower airways

The trachea, bronchi, and lungs are densely innervated by vagal afferent fibers. Those containing peptides (substance P and CGRP) originate mainly from neurons in the jugular ganglion, whereas nodose ganglion neurons innervating lower airways are almost exclusively peptide-negative (Kummer et al., 1992; Springall et al., 1987). Since the vast majority of nodose ganglion neurons binds the lectin I-B4 (Li et al., 1997), it is reasonable to assume that some of these neurons provide fibers to the lower respiratory tract.

In the rat bronchi and lungs, characteristic complex terminal structures have been shown to innervate neuroepithelial bodies (NEBs; Fig. 4F) that are thought to be a class of airway sensors for irritant stimuli (Adriaensen et al., 1998; Van Lommel et al., 1998).

3.3. Heart and aorta

In the rat heart, DiI-labeled vagal afferent terminals have been shown to consist of dense pericellular varicose endings primarily around small intensely fluorescent cells but not around principal ganglion neurons within the four major ganglionated plexuses of the epicardium, simple endings near muscle fibers of the myocardium, and more complex ‘flower-spray’ and ‘end-net’ endings in the endocardium (Cheng et al., 1997a). In addition, similar complex terminals were found in the wall of the aortic arch, and carotid artery and near glomus cells at these locations, suggesting both mechano and chemoreceptive functions (Cheng et al., 1997b). Innervation of glomus tissue within paraganglia associated with the laryngeal nerve has also been reported (Kummer and Neuhuber, 1989; Dahlqvist et al., 1994).

3.4. Thymus

Data on the vagal innervation of the thymus are scarce and controversial. Retrograde labeling of vagal brainstem neurons by tracer injections into the thymus (Bulloch and Moore, 1981; Dovas et al., 1998) have to be interpreted with great care (Nance et al., 1987). However, there might be a sparse afferent vagal innervation since data from retrograde studies (Magni et al., 1987) are corroborated by anterograde labeling of a few fibers with injections of WGA-HRP and DiI into the nodose ganglion (own unpublished observations). Sensory innervation in the absence of efferent innervation would be compatible with the idea, developed on ontogenetic and phylogenetic grounds, that the thymus anlage develops from a sensory organ located in the pharynx (Hartmann et al., 1989).

3.5. Vagal paraganglia

Paraganglia consisting of glomus cells and the occasional neuron are distributed at strategic locations along the thoracic (Kummer and Addicks, 1982; Kummer and Neuhuber, 1989; Dahlqvist et al., 1994) and abdominal vagi (Kohn, 1903; Hervonen et al., 1978; Howe et al., 1981; Böck, 1982). A particularly large number of paraganglia had been noted around the liver hilus in mice by Goormaghtigh (1936) and along the common hepatic branch and at the bifurcation of the celiac and gastric branches by Prechtl and Powley (1987). However, paraganglia are not limited to these locations and have been found in the pancreas (Berthoud and Powley, 1991) and along the periarterial nerve plexus of the celiac artery (Berthoud and Powley, 1996) (Fig. 2A). In whole mounted specimens of the hepatic arterial tree including the hepatoesophageal artery, we found an average of about 10 paraganglia (Berthoud et al., 1995a). The largest ones were typically encapsulated and associated with the fascicles of

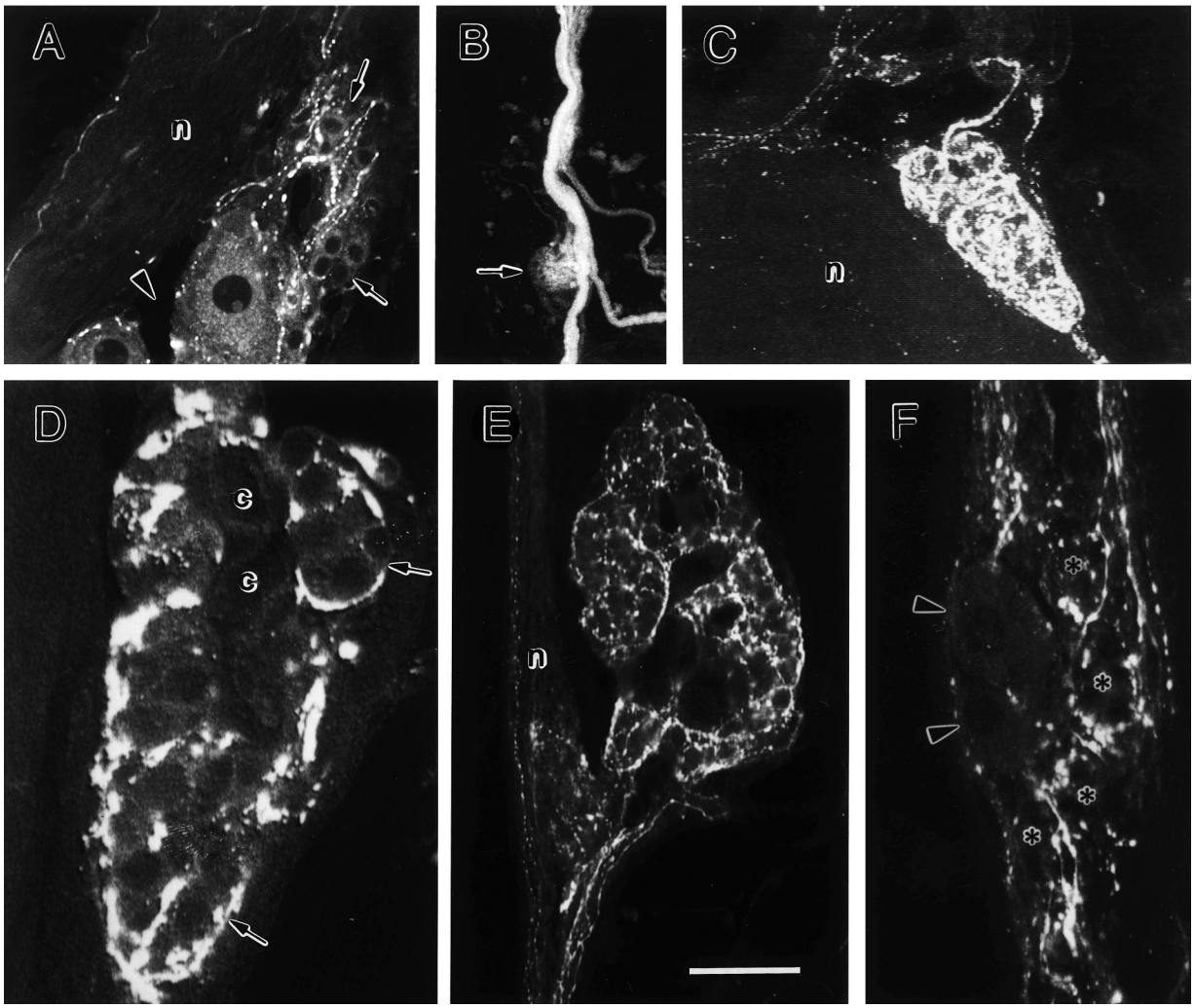


Fig. 2. Paraganglia associated with the abdominal vagus in the rat. (A) Paraganglion containing glomus cells (arrows) and neurons (arrowhead) associated with nerve bundle (n) of periarterial plexus of celiac artery is innervated by vagal efferent fibers (bright white). (B) Whole mount of common hepatic branch of abdominal vagus from rat with fluorescent tracer DiI injected into left nodose ganglion. The main nerve bundle and smaller fascicles are full of DiI-labeled axons (bright white), and a large paraganglion is innervated by vagal afferent fibers (arrow). (C, D) Paraganglion associated with common hepatic branch (n) is profusely innervated by strongly DiI-labeled axon (bright white). The confocal image in (C) is an all-in-focus image of 15 optical sections at intervals of 1.5 μm. Image in (D) is a higher magnification of a single optical section showing individual glomus cells (arrows), large capillaries (c), and cup-shaped vagal afferent terminals (bright white). (E, F) Paraganglia associated with common hepatic branch are innervated by calretinin-positive nerve fibers (bright white), which are probably of vagal afferent origin. The paraganglion in (E) is of the type forming an extrusion of the main nerve bundle (n). The one in (F) is embedded within a small nerve fascicle, and also contains two neurons (arrowheads). Glomus tissue is indicated by asterisks. Scale bar in E: 30 μm for A, E and F; 250 μm for B; 20 μm for D; 50 μm for C.

the common hepatic vagal branch (Fig. 2B–D), while smaller paraganglia, often fully embedded in nerve fascicles (Fig. 2F), were found in the liver pedicle. Nine out of 10 of these paraganglia received labeled vagal afferent terminal axons. In some of the paraganglia this innervation was very dense (Fig. 2C). Labeled axons could be seen to leave the parent nerve fascicle and to enter the paraganglionic tissue. Within the paraganglia labeled axons formed characteristic large, cup shaped varicosities, surrounding small groups of glomus cells (Fig. 2D). Double-labeled specimens showed that most glomus cells contained tyrosine hydroxylase, and that many of these catecholamine-synthesizing cells were contacted by vagal afferent terminals (Berthoud et al., 1995a). Abdominal paraganglia are also densely innervated by calretinin-positive nerve fibers (Berthoud and Patterson, 1996b; Fig. 2E and F), which are likely to be vagal afferent fibers. A sparse innervation by CGRP-positive fibers, probably of DRG origin is also present (Berthoud and Neuhuber, 1996). More recently Goehler et al. (1997) have shown that IL-1b binds specifically to glomus cells of most paraganglia in the rat.

3.6. Liver, portal vein and bile ducts

Both the HRP and DiI labeling methods allowed detailed characterization of terminal axons in the liver and associated organs. The sites of termination of vagal afferent axons are the connective tissue surrounding intrahepatic triads, extrahepatic bile ducts, portal vein, and paraganglia (Berthoud et al., 1992). In whole mounts of the hepatic artery, individual or small bundles of labeled axons from the common hepatic branch can be seen to mingle with nerve fascicles of the hepatic arterial plexus in the liver hilus. In thick sections of hepatic tissue blocks from the hilus area such labeled axons can be followed further into the liver lobes. In cross sections of interlobular portal canals, labeled fibers and terminal-like arborizations are mainly found within the walls of arteries and bile ducts (Fig. 3A). Some terminal varicose arborizations come within a few microns of surrounding hepatocytes, but the parenchyma does not seem to be the primary target (Fig. 3B). In hepatic tissue further away from the hilar area labeled vagal afferent fibers are extremely rare and always associated with triads. It cannot be ruled out completely that some vagal afferent axons penetrating into the lobular parenchyma escaped detection. While in other species such as the guinea pig, cat, monkey and human, a more or less rich parenchymal innervation was found (Forssmann and Ito, 1977; Metz and Forssmann, 1980; Tiniakos et al., 1996; Feher, 1999), this is not the case in the rat (Metz and Forssmann, 1980; Berthoud and Neuhuber, 1999). It has been suggested that because of the high concentration of gap junctions, the rat hepatic parenchyma is behaving as an ensemble, making only a few necessary neural contacts at its periphery in order to detect functional changes (Iwai et al., 1991).

Labeled vagal afferent axons traveling through the common hepatic branch and to a lesser extent through the periarterial plexus of the common hepatic artery mingle with nerve fascicles that innervate the portal vein (Berth-

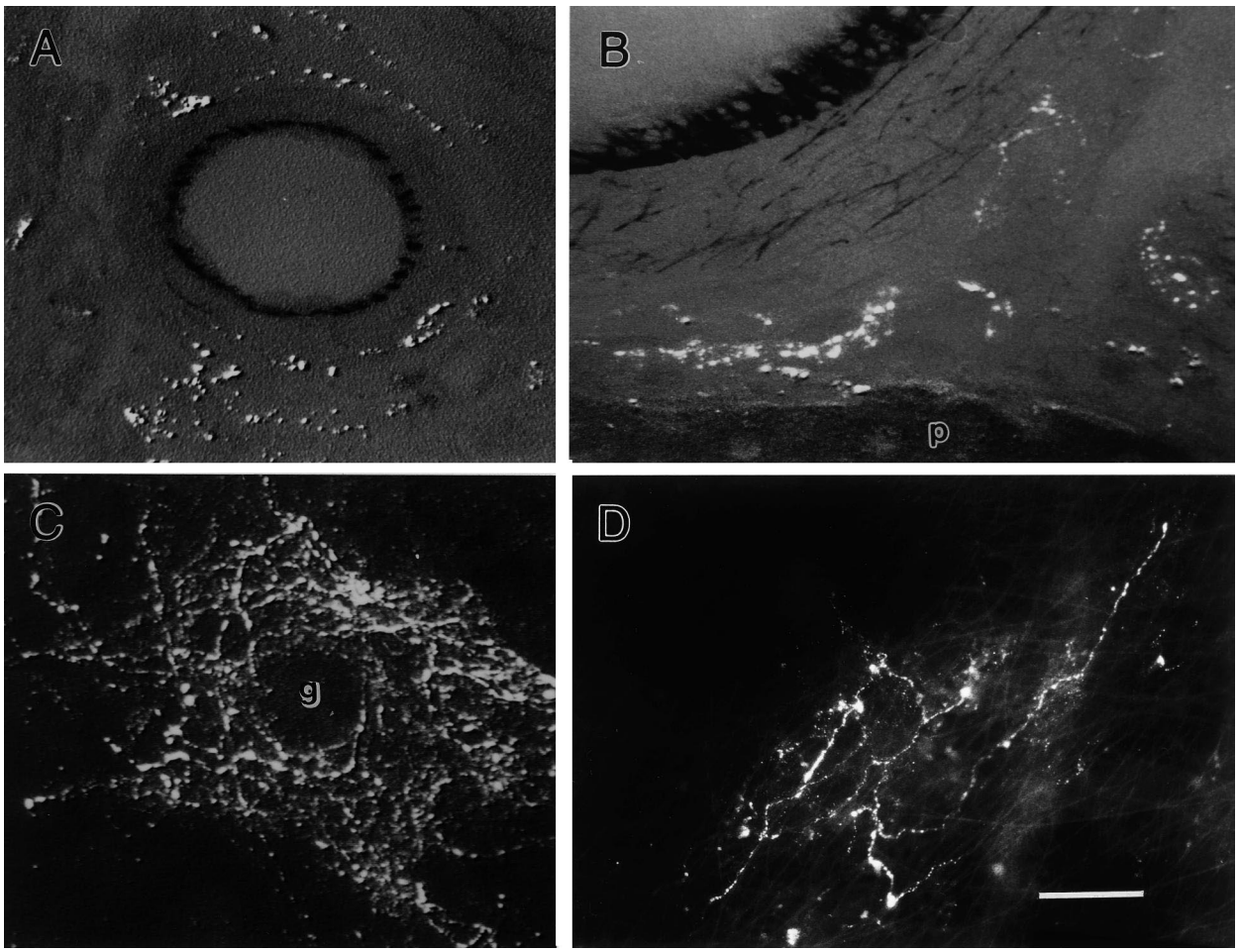


Fig. 3. Vagal afferent innervation of rat liver, bile ducts and portal vein. (A) Confocal microscope image showing DiI-labeled vagal afferent axons (bright white) surrounding artery belonging to extralobular vascular bundle. (B) Vagal afferent terminals (bright white) near hepatic parenchyma (p). No terminals were found within parenchyma. (C) Vagal afferent innervation of intrahepatic and extrahepatic bile ducts is locally very dense, with DiI-labeled terminals (bright white) swirling around peribiliary glands (g). (D) Conventional microscope image showing DiI-labeled (bright white) vagal afferent fibers in adventitia of portal vein near liver hilus. Scale bar in D: 20 μm in A, 10 μm in B, 30 μm in C, and 100 μm in D.

oud et al., 1992). Within the wall of the portal vein labeled varicose axons are found to produce terminal arborizations within both the adventitia and media (Fig. 3D). Overall such terminals are relatively rare with a tendency to be more frequent near the bifurcation of the portal vein into the two main branches supplying the left and right lobes.

As compared to vagal afferent innervation of the portal vein, innervation of bile ducts is much more pronounced and consistently detected in every specimen. Some of the fascicles containing labeled vagal afferent axons are closely attached to the major branches of the bile ducts. Labeled terminal-like structures consisting of profusely arborizing varicose fibers are located in the wall of both intra and extrahepatic bile ducts, characteristically surrounding the peribiliary glands and penetrating close to the epithelium (Berthoud et al., 1992; Fig. 3C).

3.7. Gastrointestinal tract

In the stomach, at least three distinct and characteristic terminals are present at specific locations: in both external muscle layers, in the myenteric plexus, and in the mucosal lamina propria (Fig. 4). Terminal structures in the longitudinal and circular muscle layers have been described as arrays of several long (up to a few millimetres) and rather straight axons running parallel to the respective layer, and connected by oblique or right angled short connecting branches (Berthoud and Powley, 1992; Phillips et al., 1997) (Fig. 4C). They are most frequently encountered in the longitudinal muscle layer and show a characteristic distribution with a concentration in specific areas of the fundus (Phillips and Powley, 2000).

By far the most abundant and prominent type of vagal afferent terminal is the intraganglionic laminar ending (IGLE) associated with neurons of the myenteric plexus (Fig. 4A and B (Berthoud et al., 1997a)). Because of their intimate contact with the connective tissue capsule surrounding the ganglia, one of us has suggested they represent a special class of mechanosensors in a position to detect the shearing forces between the orthogonal smooth muscle layers (Neuhuber, 1987; Neuhuber and Clerc, 1990; Neuhuber et al., 1995). This hypothesis has just recently been confirmed using electrophysiological recording from esophageal nerve fascicles that were subsequently anterogradely backfilled with biocytin (Zagorodnyuk and Brookes, 2000). However, a chemosensory role for IGLEs cannot be ruled out at present.

In the gastric mucosa, vagal afferent fibers are moderately abundant and are likely to detect the presence of luminal contents via mechanosensitivity (see below). There is no evidence for nutrient detection by gastric vagal afferents.

In the small intestine, the same three basic types of vagal afferent terminal structures have been identified by means of in vivo DiI tracing from the nodose ganglia (Berthoud et al., 1995b, 1997a). While intramuscular arrays in the longitudinal and circular smooth muscle layers are much less frequent than in the stomach (unpublished personal observation), IGLEs are abundant throughout the small (and large) intestine (Fig. 4A and B). In the absence of any other proven functional role for these endings in the myenteric plexus, and in view of the scarcity of intramuscular terminals, these IGLEs may function as intestinal tension receptors in a similar fashion as described above for the stomach and esophagus. Mucosal terminals are most abundant in the proximal duodenum, becoming relatively scarce in the distal small intestine. Vagal afferent fibers originating from the myenteric plexus penetrate the circular muscle layer and submucosa, to form networks of multiple branching axons within the lamina propria of both the crypts and villi (Fig. 4D). Terminal axons are in close contact with, but do not seem to penetrate, the basal lamina and are thus in an ideal position to detect any substances, including absorbed nutrients, and hormones and peptides released from both epithelial cells and other nerve fibers coursing through the lamina propria.

In separate studies, the anatomical relationship between mucosal mast cells and vagal afferents (Williams et al., 1997) and between CCK-containing enteroendocrine cells and vagal afferents (Berthoud and Patterson, 1996a) was inspected more closely. While in both cases, some close (<5 μm) contacts were found (Fig. 4E), there was no particular anatomical 'affinity' of vagal afferent terminals for either cell type. These findings are consistent, in principle, with a paracrine mode of action on vagal afferents of peptides, hormones and transmitters released from various sources into the mucosal lamina propria (see below). There is not much evidence for the presence of discrete and functionally specific sensory vagal axon terminals.

3.8. Vagal afferent innervation of other organs

Vagal afferent nerve terminals have also been found in rat uterus (Collins et al., 1999) and in pancreatic islets (Neuhuber, 1989). Preliminary inspection of the spleen was negative (unpublished observations and personal communication by Dr. Linda Rinaman), confirming earlier observations with retrograde tracing (Nance and Burns, 1989). Other potential sites in thoracic and abdominal organs and tissues, such as the gall bladder and adipose tissue await further investigation.

4. What is detected by vagal afferents?

4.1. Chemoreceptors including sensors for nutrients and nutrient-related compounds

In vivo recording from vagal afferent units innervating the duodenum have revealed the presence of mainly polymodal fibers in the rat (Grundy and Scratcherd, 1989),

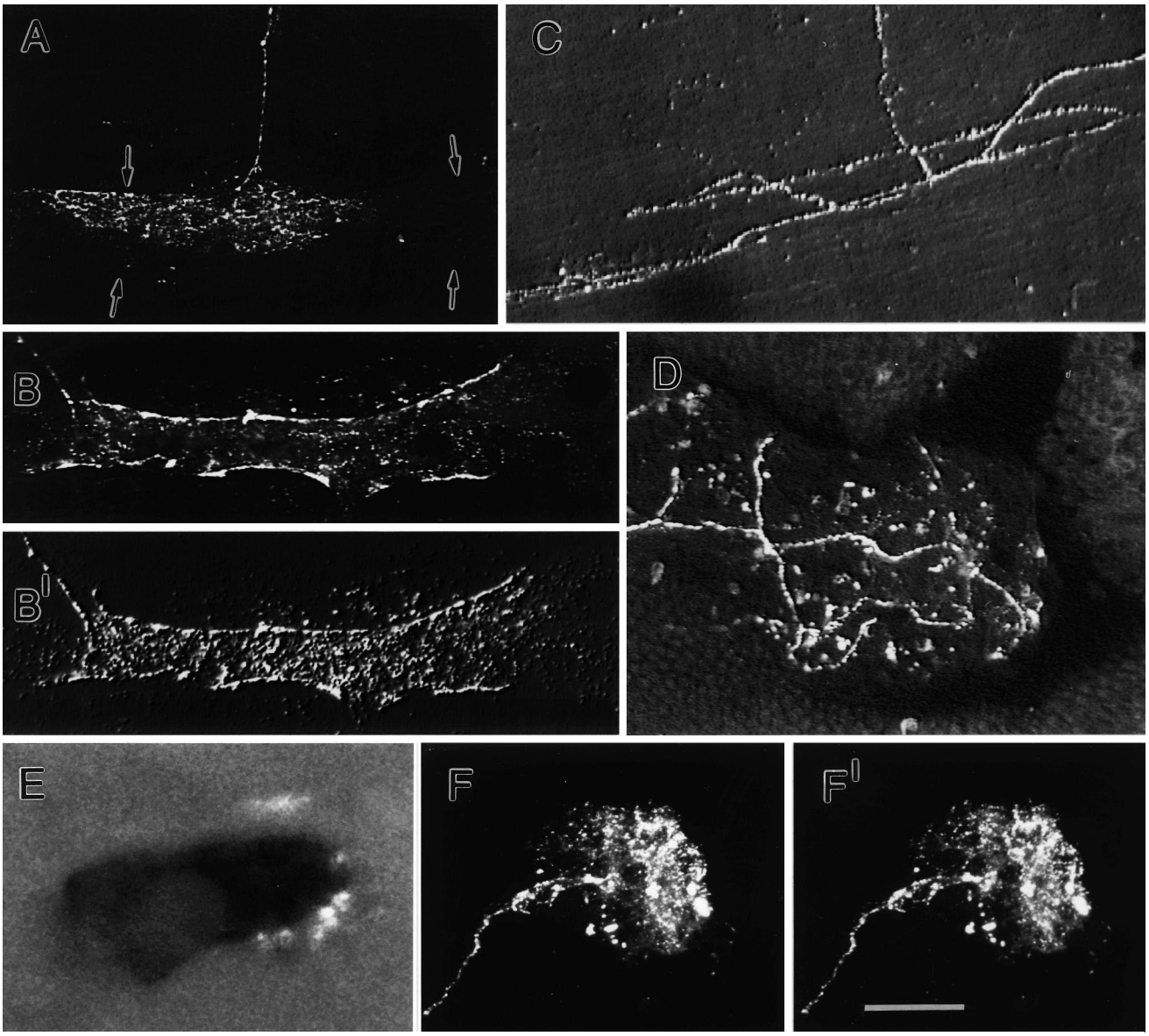


Fig. 4. Vagal afferent innervation of gastrointestinal tract (A–E) and lung (F). (A) The most abundant vagal afferent terminal structure is the intraganglionic laminar ending (IGLE) in the myenteric plexus, represented here by an example in the rat jejunum. The parent axon can be seen clearly, and the IGLE is covering about half of this large myenteric ganglion (arrows). (B, B′) The parent axon typically forms main branches on either side of the ganglion (B), from which leafy, laminar extrusions cover the ganglion in a dome-like fashion (B′), as shown by two optical sections taken at a distance of 10 μm. (C) DiI-labeled vagal afferent fiber forming an intramuscular array (bright white) in longitudinal muscle layer of rat fundus. (D) DiI-labeled vagal afferent fibers and terminals in the lamina propria near the tip of a duodenal villus. (E) Close contact of vagal afferent terminal (bright white) with mast cell (black), in mucosa of rat jejunum. (F, F′) Stereo-pair of vagal afferent terminal innervating pulmonary neuroepithelial body in rat lung. Scale bar in F′: 50 μm in A; 30 μm in B; 60 μm in C; 30 μm in D; 10 μm in E, and 50 μm in F.

and more selectively sensitive fibers in the cat (Mei, 1978). In the rat, most fibers responded to a variety of chemical stimuli applied to the lumen, such as acid, hyperosmolality and cholecystokinin (CCK), in addition to mechanical stimuli. In the cat, some fibers responded selectively to mechanical or chemical stimuli, and among chemical stimuli, selectively to glucose (Mei, 1978), amino acids, and fatty acids with different chain length (Melone, 1986). An excellent overview of this earlier literature has been summarized by Grundy and Scratcherd (1989) and by Andrews (1986). Since the advent of brain gut peptides, a number of studies have reported sensitivity of vagal afferent fibers to CCK (Davison and Clarke, 1988; Blackshaw and Grundy, 1990); 5-HT (Hillsley and Grundy, 1998a,b), somatostatin (Nakabayashi et al., 1996), IL-1β (Niijima, 1996; Kurosawa et al., 1997; Bucinskaite et al., 1997; Ek et al., 1998), and GLP-1 (Nishizawa et al., 1996, 2000).

4.2. Mechanosensors

The electrophysiological characteristics of mechanosensors in the gastrointestinal tract have been comprehensively reviewed by Grundy and Scratcherd (1989). The three major types include mucosal 'touch' receptors, muscular tension receptors, and serosal receptors. The mucosal mechanoreceptors are characterized by a low threshold for mechanical stimuli such as stroking with a fine brush, relatively rapid adaptation to continuous stimulation, and in most cases sensitivity to a variety of chemical stimuli (polymodal receptors). The so-called in-series tension receptors can be activated by stretching the external muscle layer and by spontaneous contractions, and are only slowly adapting. Because there are two distinct vagal afferent terminal structures, IGLEs and IMAs within the external muscle layer, it is presently not clear whether both endings can generate these electrical activity patterns to stretch and contractions. In the esophagus where few IMAs are present, sensitivity to circumferential stretch and probing with von Frey hairs has been localized to underlying IGLEs (Zagorodnyuk and Brookes, 2000). Thus it seems possible that IMAs are not mechanosensitive endings, but instead, as axon collaterals connected to IGLEs, may serve local effector functions.

4.3. Temperature sensors

Compared to the wealth of studies exploring chemo- and mechano-sensors, very few addressed temperature sensitivity. The first report by Nozdrachev et al. appeared in Russian. It reported increased activity as recorded with electrodes implanted into vagal and splanchnic nerve branches supplying the dog stomach upon heating the stomach. The responses were described as non-adapting and were strongest near the cardia and pylorus (Nozdrachev et al., 1977). El Ouazzani and Mei (1979, 1982) recorded from vagal afferent neurons in the cat nodose ganglia. Single units responded to cold and warm stimuli in the esophagus, gastric antrum and duodenum but apparently not to mechanical and chemical stimuli. The authors called these units true thermoreceptors. Adachi (1984) reported that activity of vagal afferent fibers dissected from the rat common hepatic branch correlated to liver temperature and Cottrell and Iggo (1984) reported cold-sensitivity of some mechanoreceptors with afferent C-fibers in the sheep duodenum. It should be noted that a population of capsaicin-sensitive DRG sensory neurons are also sensitive to elevated temperatures, and are likely responsible for heat hyperalgesia (Guenther et al., 1999).

None of the transduction mechanism(s) involved in these temperature-sensitive vagal afferents has been studied in detail. Furthermore, the exact location and morphological type of vagal afferents involved in these studies remains unknown.

4.4. Osmosensors

Adachi reported two types of osmosensitive fibers in the common hepatic branch of the vagus nerve; one characterized by increasing frequency of spike discharge in response to higher osmotic pressure, the other with the same response to decreasing osmotic pressure (Adachi, 1984; Adachi et al., 1996). While the group of Mei in the cat (Mei and Garnier, 1986) and Blackshaw in the ferret (Blackshaw and Grundy, 1990) found vagal afferent units in the duodenum that responded to osmotic stimuli to be polymodal in nature, Cottrell and Iggo (1984) did not find any of the identified mechano- and/or chemosensitive units in sheep duodenum to be osmosensitive.

4.5. Vagal afferents and nociception

Although it is commonly assumed that vagal afferents are not involved in nociception and pain, there is growing evidence that they play a complex role in these processes. High threshold distension sensitive gastric vagal afferents have been characterized in the rat (Sengupta et al., 1993). 'Scratching', 'tearing' or 'burning' sensations of irritation of lower airways are transmitted mainly through vagal afferents (Guz, 1977; Morton et al., 1951). Vagal afferents from the upper aerodigestive tract terminate profusely in the paratrigeminal nucleus which is thought to be involved in nociceptive processing (Altschuler et al., 1989; Saxon and Hopkins, 1998). Spinothalamic tract neurons in the upper cervical spinal cord are often excited by electrical stimulation of vagal afferents (Fu et al., 1992; Chandler et al., 1996). Direct projections of primary vagal afferents to the upper cervical spinal cord have been demonstrated also in tracing studies (McNeill et al., 1991). Noxious gastric distention resulted in c-fos expression in the nucleus of the solitary tract which is even more pronounced than that in the spinal cord. This c-fos expression could be largely ascribed to activation of vagal afferents and to a minor extent activation of a spino-solitary pathway (Traub et al., 1996). Esophageal distension has been shown to elicit cardiovascular reflex responses mediated by vagal afferents (Loomis et al., 1997; Dong et al., 2000), and this has been interpreted as a nociceptive event. Vagal afferents may contribute to the affective-emotional rather than to the sensory-discriminative aspect of pain (Traub et al., 1996). Furthermore, vagal cardiac afferents express adenosine A1 receptors which may be activated during ischemia and mediate cardiac pain (Middlekauff et al., 1998).

There is also a considerable body of evidence that vagal afferents are able to exert both inhibitory and excitatory modulation of spinal nociceptive processing via bulbospinal pathways (Ammons et al., 1983; Randich and Gebhart, 1992; Hummel et al., 1997). There is even a sophisticated modulatory action of vagal afferents in the celiac branch on bradykinin-induced plasma extravasation and hyperalgesia in the rat knee joint (Miao et al., 1994,

1996; Khasar et al., 1998a). This is mediated on the efferent side by a hormonal signal from the adrenal medulla (Khasar et al., 1998b). Finally, and most relevant to the overall theme of immunosensation, Watkins et al. (1994a,b) have demonstrated that LPS- and IL1β-induced hyperalgesia depends on the integrity of the vagus nerve, particularly its common hepatic branch.

4.6. Transduction mechanisms

Several investigators have speculated about the transduction mechanisms involved in vagal afferent sensors (Mei, 1978; Grundy and Scratcherd, 1989). However, because of the poor accessibility of most sensors the process of impulse initiation has not yet been directly addressed. Analogies can be and have been drawn to gustatory sensation. In taste buds, chemical stimuli do not directly activate sensory nerve endings, but act on specific receptors and/or ion channels of taste receptor cells. A variety of intracellular signaling cascades then ultimately lead to the release of transmitters and peptides from the taste receptor cells on afferent nerve terminals. The presence of enteroendocrine cells capable of releasing transmitters and peptides upon luminal nutrient stimulation and of proteins specifically involved in taste transduction such as gustducin in enterocytes support such analogies (Hofer et al., 1996). In addition, mechanical and chemical stimuli could directly activate free nerve endings through specific receptors and/or mechanosensitive ion channels.

5. Selective interruption of vagal afferents

5.1. Vagal afferent rhizotomy

In the cat, vagal efferent fibers are relatively well separated from ganglion cells and their afferent fibers, and a procedure has been described to selectively interrupt efferent or afferent fibers at this more easily accessible level (Rodrigo et al., 1982). In the rat this is not possible. However, vagal afferent fibers enter the caudal medulla at a more dorsal level than efferent fibers leave the medulla (Fig. 1). Thus, methods have been developed to selectively transect either afferent or efferent rootlets (rhizom) intracranially. Both a ventral (Norgren and Smith, 1994) and a dorsal (Walls et al., 1995) approach have been used. It could generally be said that the ventral approach lends itself better for efferent and the dorsal approach for afferent rhizotomy, because the respective rootlets to be cut are closer to the surface. Also, because of the relatively difficult surgical procedure including drilling a rather large hole into the cranium, the method has only been applied unilaterally. Unilateral rhizotomy is typically combined with contralateral subdiaphragmatic vagal trunk or branch cuts. Thus, the procedure is not completely selective in that subdiaphragmatic efferents are only unilaterally spared. More importantly, because it is difficult to localize the thoracic vagal branches, such surgery would leave one half of the afferent fibers innervating lungs, heart, paraganglia, etc. intact. These facts need to be considered when experimental results are interpreted.

5.2. Pharmacological deafferentation by capsaicin

Systemic treatment of neonatal and adult rats with capsaicin destroys a class of thin, unmyelinated primary afferents of both DRG and nodosal origin (Jancso et al., 1977; Nagy et al., 1981; Chung et al., 1990; Holzer, 1991; Carobi, 1996). The effect is due to the presence of the VR-1 vanilloid receptor on these afferent neuron populations (Michael and Priestley, 1999). Binding of capsaicin to the receptor and thus neurotoxicity can be blocked by the vanilloid receptor antagonist capsazepine (Szallasi, 1995). Local application of capsaicin to the vagal nerve trunks (perivagal capsaicin treatment) has been found to make vagal afferents non-functional and might represent the best method for selective vagal deafferentation (Lloyd et al., 1993). However, not all vagal afferents seem to be capsaicin-sensitive. We found that many vagal afferent fibers innervating esophagus and stomach could still be anterogradely traced with DiI in rats that underwent prior systemic capsaicin-treatment (Berthoud et al., 1997a). At the functional level, gastric distension was still capable of inducing Fos expression (Berthoud et al., 1997b) and electrical activity (Ritter et al., 1989) in NTS neurons. Schuligoi et al. (1998) reported that gastric acid-evoked c-fos expression in the rat brainstem is mediated by capsaicin-resistant vagal afferents. On the other hand, Carobi found a dramatic loss of neurons in the nodose ganglion in neonatally capsaicin treated rats (Carobi, 1996), thus supporting earlier in vitro studies (Marsh et al., 1987), and reported the liver (Carobi and Magni, 1985) and pancreas (Carobi, 1987) to be innervated by capsaicin-sensitive vagal afferents. Likewise, capsaicin-sensitive vagal afferents seem to be involved in 5-HT3-receptor-mediated mast cell degranulation and c-fos expression in various brain nuclei after intestinal anaphylaxis (Castex et al., 1994, 1995).

In addition to capsaicin treatment, kainic acid injection into the nodose ganglia has been used to selectively destroy sensory perikarya but not efferent fibers of passage (Lewis et al., 1990).

5.3. Hepatic branch vagotomy

With particular relevance for this special issue, hepatic branch vagotomy has been used extensively with the aim to vagally deafferent the liver. There are four problems with this intention. Firstly, the fact that the common hepatic branch innervates not only the liver but also parts of the stomach, pyloric sphincter, pancreas and proximal duodenum has been completely ignored when behavioral

and or physiological experiments using hepatic branch vagotomies were interpreted. Behavioral changes following hepatic branch cuts do not implicate vagal fibers supplying the liver more than they implicate vagal fibers supplying duodenum and pancreas by the gastroduodenal branch. Furthermore, changes of specific physiological hepatic parameters (such as enzyme activities) following hepatic branch vagotomies do not necessarily result from cutting fibers to the liver, but could be the result of, or an interaction with, changes that occur 'upstream' in the simultaneously denervated duodenum or pancreas. In order to avoid these problems, vagotomies will have to become more selective, or alternative approaches have to be developed. While it will be extremely difficult to carry out selective transections of the hepatic branch proper (only the vagal fibers that terminate in the liver, and not to mention selective transection of vagal afferent fibers in this branch), cutting the gastroduodenal vagal branch should be routinely used as a control procedure for common hepatic branch vagotomies.

Secondly, some of the afferent vagal innervation of the liver originates in the right nodose ganglion and projects through the dorsal subdiaphragmatic trunk, the dorsal celiac branch, and the periarterial plexus of the common and proper hepatic arteries (Berthoud et al., 1992; Phillips et al., 1997). The existence of this pathway had been shown earlier by electrophysiological techniques (Niijima, 1983). This projection cannot be easily cut in a selective manner, and although the contribution of afferent fibers to the liver by this route is only about 10%, it cannot be completely ignored. For example, lack of longer term changes in hepatic functions following common hepatic branch vagotomies cannot be taken as evidence against vagal involvement in this function, since the remaining fibers traveling through this dorsal route could have sprouted and taken over some function.

Thirdly, because about 30% of the fibers in the common hepatic branch are of non-vagal origin (Prechtl and Powley, 1990), any effect of hepatic vagotomy could be attributed to ablation of these adventitial fibers. These could be sympathetic postganglionic or dorsal root afferent fibers.

Fourthly, although only about 10% of vagal fibers in the hepatic branch are efferent, this cannot be completely ignored in the interpretation of physiological and behavioral results.

6. Central vagal afferent signaling

6.1. Anatomical and topological segregation of primary afferent vagal input to the dorsal medulla

Central terminals of primary vagal afferents are mainly found in the nucleus tractus solitarius (NTS) and the area postrema, fewer in the dorsal motor nucleus of the vagus and in the trigeminal islands. The best viscerotopic segregation is between cardiac and pulmonary afferents in the lateral subnuclei of the NTS, and the alimentary canal afferents in the medial subnuclei, the area postrema and the dorsal motor nucleus. Within the alimentary canal afferents there is a general rostro-caudal viscerotopy, with taste input from the tongue most rostral, and intestinal input most caudal (Kalia and Mesulam, 1980a,b; Norgren and Smith, 1988; Altschuler et al., 1989). This is based on experiments using retrograde tracer injections into various abdominal vagal branches or viscera and mapping of vagal afferent terminals. However, because such tracer injections cannot be limited to specific tissue compartments or receptive sites in the periphery and because there is no tight anatomical relationship between terminal density and second order neurons, this method can only provide crude viscerotopic maps and is not adequate to investigate sensory pathways with functional or sensory specificity.

In general there is little information available regarding the central dissemination of functionally specific sensory input from various abdominal vagal mechano-, nutrient- and metabolic sensors. Besides a few electrophysiological studies with usually a limited number of neurons tested (Barber and Burks, 1983; Chambert et al., 1993), such information comes almost exclusively from experiments using immediate-early gene expression (Fig. 5A–C). Treatments that inhibited food intake like i.p. CCK, hypertonic saline, and feeding after deprivation produced different patterns of c-fos expression in the dorsal medulla than treatments that stimulate food intake, like food deprivation and i.p. insulin (Olson et al., 1993; Rinaman et al., 1993; Fraser and Davison, 1993). Furthermore, the experimentally separated cephalic, esophageal, and gastric phases of meal ingestion also generated different patterns of c-fos expression (Emond and Weingarten, 1994; Fraser et al., 1995). Finally, direct gastric balloon distension (Willing and Berthoud, 1997; Fig. 5B) and intestinal infusion of various nutrients (Ritter et al., 1992; Zittel et al., 1994; Phifer and Berthoud, 1998; Fig. 5C) produced more or less distinct patterns of c-fos expression in the dorsal medulla.

6.2. Neurotransmitters used by primary vagal afferents

Many of the vagal afferent neurons residing in the jugular ganglion contain peptides such as CGRP and substance P. Much fewer sensory neurons in the nodose ganglia contain peptides (Zhuo et al., 1997; Ichikawa and Helke, 1997). There is strong evidence for L-glutamate acting through both NMDA and non-NMDA ionotropic glutamate receptors in cardiovascular vagal afferents (see Talman (1994) for a review). Similarly non-NMDA glutamatergic receptor blockers have been shown to block all taste input to the NTS (Li and Smith, 1997). Whether glutamate plays an equally dominant role in vagal afferents from the abdominal viscera is not clear. In one study,

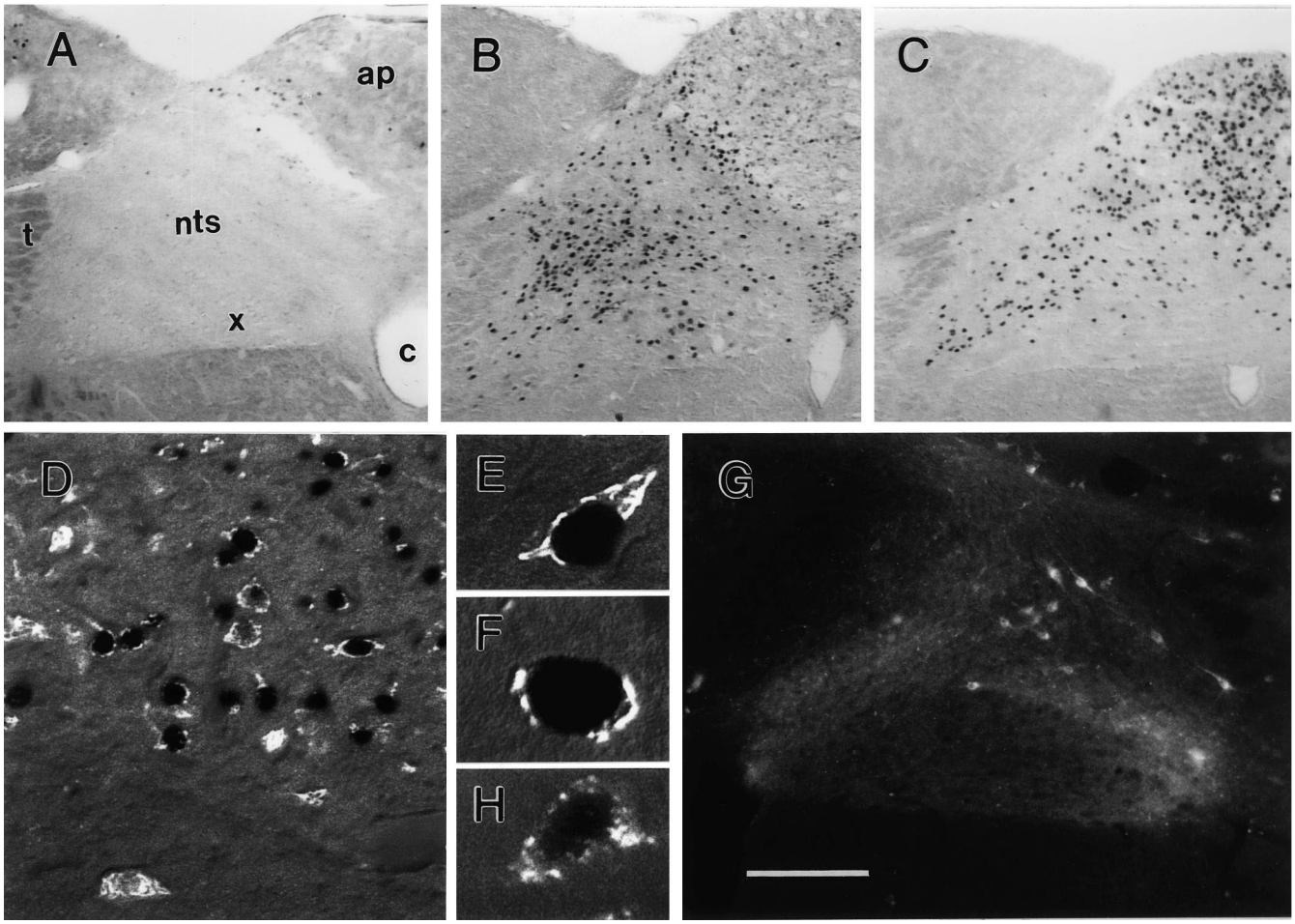


Fig. 5. Topography and neurochemistry of vagal afferent pathways in the brain. (A–C) Induction of c-Fos expression in dorsal vagal complex by specific sensory stimuli such as infusion of saline (A) or glucose (C) into the duodenum, or gastric balloon distension (B) in awake, freely moving rats bearing chronic intraduodenal catheters and gastric fistulas. Note that in the control condition (A) very few neurons show Fos expression, and that the selective stimuli produce different patterns, with Fos expression in the NTS, area postrema, and the dorsal motor nucleus. (D–F) Identification of NMDA receptor on NTS neurons activated by gastric balloon distension by using double immunohistochemistry for Fos and NR1. The lower magnification confocal image in (D) shows that a large percentage of activated neurons (black nuclei) express the NMDA receptor (bright white), and two examples of receptor-bearing, activated neurons are shown at higher magnification in (E, F). (G, H) Assessment of projections of gastric distension-activated NTS neurons by using retrograde tracing from paraventricular nucleus of the hypothalamus (PVN). The low magnification conventional image in (G) shows retrogradely labeled neurons (bright white) in the NTS on the background of DiI-labeled axon terminals (pale white) resulting from injection into ipsilateral nodose ganglion. A neuron that is activated by gastric distension (black, Fos-labeled nucleus) and projects to the PVN (retrograde tracer in cytoplasm, bright white) is shown at higher magnification in (H). Abbreviations: ap, area postrema; c, central canal; nts, solitary nucleus; t, solitary tract; X, dorsal motor nucleus of vagus. Scale bar in G: 200 μm in A–C; 30 μm in D; 120 μm in G, 12 μm in E, F, H.

excitatory input to NTS neurons was blocked by local microinjection of the general glutamatergic blocker kynurenic acid (McCann and Rogers, 1994). However, in another study, local perfusion with K^+, but not electrical stimulation of the cervical vagus increased glutamate concentration in an NTS dialysate, questioning glutamate as the major transmitter (Sved and Curtis, 1993). Also, gastric distension-induced Fos expression in the NTS was only partially reduced by 4th ventricular injection of the NMDA receptor antagonist MK-801 (Zheng et al., 1999).

There is histological and pharmacological evidence for various glutamate receptor subtypes in the dorsal vagal complex (Dutschmann et al., 1998; Aicher et al., 1999; Berthoud et al., 2000; Fig. 5D–F). The ionotropic NMDA receptor subtype NMDA-R1 has been localized quite selectively to esophageal premotor neurons in the central subnucleus of the NTS (Broussard et al., 1994). We have found NR2 immunoreactivity associated with about 60–70% of NTS neurons activated by gastric balloon distension (Fig. 5D). There is also pharmacological evidence for the non-NMDA ionotropic ampa/kainate receptor (Willis et al., 1996), and we have found GluR1 immunoreactivity in about 30–40% of NTS neurons activated by gastric distension (Berthoud et al., 2000).

7. Functions of vagal afferent sensors

7.1. Local effector functions

There is now convincing evidence that dorsal root spinal afferents are capable of local effector functions via axon collateral reflexes and the release of peptides such as calcitonin gene-related peptide and substance P (Holzer, 1991, 1998). Because only a small proportion of vagal afferents contain these two peptides, it is not clear whether they can serve similar local effector functions. Vagal afferent fibers in the gastrointestinal tract generally exhibit complex axonal projections and terminal structures, and the presence of axon collaterals has been shown in a few cases. Furthermore, synaptic contacts between IGLEs and myenteric neurons have been described at the ultrastructural level (Neuhuber, 1987; Neuhuber and Clerc, 1990) and substance P immunoreactivity has been colocalized in identified IGLEs of the esophagus (Kressel and Radespiel-Tröger, 1999). At the functional level, vagal-stimulation-induced gastric motility responses have been described in hexamethonium-treated rats (Delbro et al., 1983) as well as in supranodose vagotomized cats (Tsubomura et al., 1987), and capsaicin-sensitive afferent fibers contributed to vagal stimulation-induced changes in gastric mucosal blood flow (Thiefin et al., 1990).

On the other hand, antidromic stimulation of the cervical vagus induced c-Fos expression in only a very small ($<$1%) proportion of gastric myenteric plexus neurons, while stimulation of vagal efferents induced Fos in most enteric neurons (Zheng et al., 1997), and it did not induce intestinal motility responses in dogs (Neya et al., 1990).

7.2. Central neural effects

Neurons that receive synaptic input from terminals of primary afferents can be categorized as second order sensory neurons. They, in turn, have axonal projections to third order neurons (Fig. 1). The major outputs from NTS include: (1) local projections to medullary motor nuclei such as the vagal dorsal motor nucleus (dmnX), nucleus ambiguus (amb), and motor V nucleus as well as to the rostral ventrolateral medulla (RVLM) including the A1 noradrenergic cell group and other brainstem interneurons in areas also referred to as reticular formation. (2) Strong projections to the parabrachial/Koelliker-Fuse nuclear complex in the dorsolateral pons. (3) Ascending projections to forebrain sites including various parts of the hypothalamus (PVN, LHA, ARC, DMN) (Fig. 5G and H), amygdala, bed nucleus of the stria terminalis, and insular cortex (Ter Horst et al., 1989).

Given these anatomical connections, signals generated in vagal afferents have the potential to affect the entire organism. Relatively few specific functions have been well characterized. It has been suggested by Lawes and comrades (Andrews and Lawes, 1992; Leslie et al., 1992) that vagal afferents have a generally protective role and were originally (in lower vertebrates) projecting exclusively to the paraventricular system, a collection of subependymal and subpial nuclei that once served protective and defensive somatic reflexes. According to Lawes, this primordial function is exemplified by the lateral line system in fish. In these earlier life forms control of homeostatic functions by the central nervous system was limited to escape and avoidance of perturbing stimuli or suboptimal conditions, because autonomic innervation of peripheral organs was limited (Andrews and Lawes, 1992). As the internal milieu came more under the control of the autonomic and central nervous systems, these same pathways in the paraventricular system were used for the elaboration of vago-vagal and vago-spinal reflexes serving homeostatic control. Andrews and Lawes suggested ‘great wandering protector’ would be an adequate name for the vagus nerve.

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