Review

The distribution and chemical coding of neurons supplying the sphincter of Oddi in mammals

O. Tomaszewska, J. Kaleczyc

Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 13, 10-718 Olsztyn, Poland

Abstract

The major duodenal papilla (papilla of Vater) is an important structure associated with the biliary tract and, in some species, the pancreas. It usually represents a slight elevation on the intestinal mucosa where the dilated junction (ampulla of Vater) of the common bile duct and pancreatic duct enters the duodenum. The ampulla is surrounded by a specifically arranged muscle structure called the sphincter of Oddi (SO) which controls the flow of bile and pancreatic fluid. The function of the sphincter is regulated by a complex system that involves many hormonal and neural factors. The literature in the field contains detailed data on the morphology of the SO in a number of mammalian species. However, the comprehensive information about the anatomy and neurochemistry of the innervation of this structure is very limited. The present review article summarizes the current knowledge on the innervation of the SO in mammals. Special emphasis has been put on the localization and chemical coding of neurons contributing to this nerve supply.

Key words: major duodenal papilla, sphincter of Oddi, innervation, neurons, neuronal tracing, immunohistochemistry, neurotransmitter markers

Introduction

The major duodenal papilla (MDP; papilla of Vater) is an important structure associated with the biliary tract and, in some species, the pancreas. The anatomy of the MDP is relatively well recognized, especially in laboratory mammals and man, although its structural organization differs considerably between species. Nevertheless, it usually represents a slight elevation on the intestinal mucosa where the dilated junction (ampulla of Vater) of the common bile duct (CBD) and pancreatic duct (PD) enters the duodenum (Dodds 1989, Toouli and Baker 1991, Padbury et al. 1993a, Horiquchi and Kamisawa 2010). The ampulla is surrounded by a specifically arranged muscle structure called the sphincter of Oddi (SO; sphincter of ampulla). In species possessing the accessory pancreatic duct only, the sphincter is associated with the final segment of the bile duct. The physiological role played by the SO is fine and complex, because it not only controls the flow of bile and pancreatic juice into the duodenum, but also prevents the reflux of duo-
The function of the sphincter is regulated by a complex system that involves many hormonal and neural factors (Sarles 1986, Grace et al. 1990, Woods and Sacccone 2007). As already mentioned, the literature in the field contains detailed data on the morphology of the SO in a number of mammalian species. However, the comprehensive information about the anatomy and neurochemistry of the innervation of this structure is very limited.

The present review article summarizes the current knowledge on the innervation of the SO. Special emphasis has been put on the localization and chemical coding of neurons contributing to this nerve supply.

### General remarks on the innervation of the gastrointestinal tract

The innervation of the gastrointestinal tract is very complex and differs from that of many other viscera. It is primarily accomplished by numerous neurons forming intramural ganglia. These so called enteric neurons are found not only in the wall of the oesophagus, stomach and gut but also in the wall of the gallbladder as well as the cystic and biliary duct (Cai and Gabella 1983a, Mawe and Gershon 1989, Mawe et al. 1997). Some of them are distributed within the MDP and SO, and supply not only the sphincter but also project outside the papilla to innervate the duodenal tissues. In addition to the intramural neurons, neurons found in sympathetic pre- and paravertebral ganglia, and in sensory vagal and dorsal root ganglia contribute to the innervation of organs in the gastrointestinal tract.

The following paragraphs briefly summarise the current knowledge on the intrinsic (intramural) and extrinsic innervation of the SO.

### Intrinsic innervation of the sphincter of Oddi (Fig. 1)

The precise analysis of the literature in the field suggests that the intrinsic innervation of the SO is accomplished by two categories of intramural duodenal neurons: those which are located within the MDP/SO, and those, which are distributed outside the MDP/SO within the duodenal intramural ganglia. Unfortunately, the detailed information dealing with the distribution of these neuronal populations is rather limited.

Ganglionated plexuses distributed between the muscles of the SO have been described in detail by Padbury et al. (1993a) in the Australian brush tailed possum. The authors used anti-neuron specific enolase (NSE) and anti-S100 immunohistochemistry to visualize neurons and anti-desmin antibodies for muscle identification. In the possum, the external sphincteric plexus is located between the longitudinal and circular muscle layers of the external sphincter muscle, and is connected with the myenteric plexus of the duodenum. Another, intersphincteric ganglionated plexus is found between the external and internal sphincter musculature. The internal sphincteric plexus is located between the circular and longitudinal muscle layers of the internal sphincter. It has to be mentioned that ganglia are also distributed in the muscular septum between the biliary and pancreatic duct, and the internal sphincteric plexus is continuous with the plexus of the septal ganglia. At the duodenal end of the sphincter, as it passes through the submucosal portion of the duodenal wall where the inner circular muscle of the internal sphincter disappear, ganglia of the intersphincteric and the internal sphincteric plexus intermingle to form another the ampullary plexus.

Simula et al. (2001) have revealed that the number of neurons associated with the possum SO is significantly higher within the distal region (duodenal end) of the sphincter than in the more proximal part. Furthermore, they found that approximately (approx.) 50% of all SO associated nerve cells were immunoreactive to nitric oxide synthase (NOS; marker of nitrergic nerve structures) and that 27% of these nitrergic neurons displayed immunoreactivity to vasoactive intestinal polypeptide (VIP). It should be mentioned, that in the myenteric plexus of the duodenum, only approx. 25% of neurons exhibited immunoreactivity to NOS. NOS- and VIP-positive varicos nerve fibres were present not only within the nerve plexuses of the duodenum and SO, but also in the circular and longitudinal muscle layers.

Padbury et al. (1993b) have also applied retrograde tracing method combined with immunohistochemistry to investigate the distribution and chemical coding of intramural duodenal neurons projecting to the SO in the same species (the authors have also investigated the distribution of the retrogradely labeled neurons in extrinsic autonomic and sensory ganglia; these data are analyzed in the next chapter). The authors have used two fluorescent neuronal tracers, Fast Blue (FB) and 1,1′-dioctadecyl-3,3,3′,tetramethylindocarbocyanine perchlorate (DiI). In some possums, FB or DiI, respectively, was injected into the extraduodenal part of the SO while in some other animals both dyes were separately injected. In all the animals, retrogradely labeled neurons were found in...
the submucous and myenteric plexus both proximal and distal to the MDP. However, in the submucous plexus the neurons were much less frequent (only single nerve cells were encountered) than in the myenteric plexus. In the myenteric plexus, the frequency of the labeled neurons (a total of 134 labeled nerve cells have been found in a single animal after sphincter injections) decreased with the distance from the MDP and extended from the pyloro-duodenal junction to 29 mm distal to the sphincter/duodenal junction. Immunohistochemistry revealed that about one-third of the retrogradely labeled neurons were immunoreactive for enkephalin (ENK).

The distribution and chemical features of intrinsic neurons supplying the SO in the Australian brush tailed possum have been investigated also by Simula et al. (1997). These authors have also applied retrograde tracing method with the use of a fluorescent tracer DiI (administered to the distal SO circular muscle) in combination with immunohistochemistry. They found 329 DiI labeled neurons in both the duodenal myenteric ganglia and in the ganglia located within the MDP, associated with SO muscles. The majority of the myenteric neurons were distributed within a relatively small area surrounding the place of the dye injection (within a distance of about 5 mm from the injection site). This study has also revealed that many (approx. 26.5% or 8.8%, respectively) duodenal SO-projecting myenteric neurons contained immunoreactivity to choline acetyltransferase (ChAT; marker of cholinergic nerve structures) or NOS. Some (approx. 5.5%) retrogradely labeled neurons exhibited colocalized immunoreactivities to both enzymes. On the other hand, the majority (approx. 54.1%) of the DiI-positive neurons located in the SO plexus were NOS-immunoreactive, and some of them (approx. 11.5%) revealed immunoreactivity to ChAT. A small population (approx. 4.1%) of the retrogradely labeled neurons contained simultaneously immunoreactivities to both ChAT and NOS. The authors have concluded that the distal SO circular muscle in the possum receives direct inputs from intrinsic neurons found within the myenteric plexus of the duodenum and SO. The putative excitatory motor cholinergic (ChAT-positive) neurons are distributed predominantly within the myenteric plexus and the presumably inhibitory motor nitrergic (NOS-positive) neurons are found within the SO associated local plexus. The large (nearly 60%) component of ChAT/NOS-negative neurons may be indicative of a unique motor function of the sphincter.

Ganglia distributed between the SO muscles have been also well described and immunohistochemically characterized in the guinea pig (Cai and Gabella 1983a, Wells and Mawe 1993, Wells et al. 1995, Talmage et al. 1997, Kennedy and Mawe 1998, Mawe and Kennedy 1999). Cai and Gabella (1983a) have used histochemical methods for demonstration of adrenergic and cholinergic nerve structures to investigate the innervation of the gall bladder and biliary pathways. They have found that the ganglionated plexus associated with the CBD splits into two interconnected components at the level of the lower portion of the duct to the duodenum, where there is a conspicuous increase in the thickness of the muscle coat. One plexus is situated close to the inner surface of the muscle coat, the other, much more extensive, has intramuscular localization. The inner plexus is morphologically similar to the plexus of the gall bladder and CBD, and appears in continuity with it and with the submucosal plexus of the duodenum. The intramuscular component is in continuity with the myenteric plexus of the duodenum.

Similar results dealing with the morphological arrangement of the intrinsic innervation of the guinea pig SO have been obtained by Wells and Mawe (1993) who have performed immunohistochemical investigations on the whole mount preparations with the use of a neuronal marker NSE. These authors have found that the ganglionated plexus of the SO resembles the myenteric plexus of the small intestine and includes a network of approx. 50 ganglia, each containing approx. 54 neurons (range 5-181 cells/ganglion) and interganglionic fiber bundles.

Immunohistochemical and histochemical studies performed by Wells et al. (1995) have revealed that neurons in guinea pig SO ganglia express immunoreactivity for several biologically active substances including substance P (SP), enkephalin and/or β-endorphin (ENK-END), neuropeptide Y (NPY), VIP, calcitonin gene-related peptide (CGRP) and NOS. NOS immunoreactivity was found in a subpopulation of neurons which also displayed nictinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) activity. Immunoreactivity for SP, ENK-END, NPY, VIP, CGRP and NOS was also expressed by intraganglionic nerve fibres and nerve terminals distributed within major interganglionic connections and the tertiary plexus. The two most widespread immunoreactivities detected in the SO neurons were those for SP and ENK-END. Neurons that were immunoreactive for SP and/or ENK-END comprised approx. 57% of the total neuronal population. Of the total population, approx. 39% were double labeled for both substances. Of the cells that were not double labeled, SP- or ENK-END-positive neurons comprised approx. 7% or 14%, respectively. Neurons displaying NADPH-d activity and NOS immunoreactivity represented the next most common, distinct from the SP/ENK-END neurons, population (approx. 27%) of nerve cells in the SO. Small popula-
The distribution and chemical coding of neurons in the SO were immunoreactive for NPY and VIP (approx. 12% and 10%, respectively). It has appeared that neurons immunoreactive to these neuropeptides are subpopulations of the NOS-positive nerve cells. The SO neurons immunoreactive for CGRP were seldom observed. However, CGRP-IR nerve fibres were very abundant in the SO ganglionated plexus. Some of them expressed simultaneously immunoreactivity for SP, and many SP-positive only nerve terminals were also found within the ganglia and in nerve bundles closely associated with the SO muscle. These CGRP and/or SP-positive fibres have been suggested by the authors to represent sensory nerve terminals of extrinsic origin.

Talmage et al. (1997) have performed investigations dealing with the identification of cholinergic neurons in guinea pig SO ganglia, thus complementary to those carried out by Wells et al. (1995). Based on the results of these two studies it can be concluded that about 69% of the neurons within the guinea pig SO ganglia are immunoreactive for ChAT thus can be classified as cholinergic nerve cells (Talmage et al. 1997). Most of these neurons coexpress SP and/or ENK-END, and they have been shown to increase SO tone (Behar and Biancani 1984, Dahlstrand et al. 1985, Dahlstrand et al. 1988). Another population, consisting of approx. 27% of the neurons, comprises nerve cells expressing immunoreactivity to nitric oxide (NO) and NADPH-d activity. This population of nitrergic neurons includes two subgroups, one which contains immunoreactivity to VIP and the other which is NPY-positive. Nitric oxide and VIP have been revealed to decrease SO tone (Dahlstrand et al. 1989a,b, Kaufman et al. 1993, Pautetzki et al. 1993). It should be emphasized, that in the guinea pig, SO cholinergic and nitrergic neurons represent separate populations of nerve cells, because no neurons containing colocalized cholinergic and nitrergic markers have been observed (Hillsley and Mawe 1998a). These findings along with results indicating that the majority of neurons in the SO are motor nerve cells (Wells and Mawe 1993) suggest that the two populations of the SO neurons represent excitatory and inhibitory motor neurons, respectively.

It should be mentioned that the guinea pig SO possesses also an extensive serotonergic innervation (Hillsley and Mawe 1998b). The SO ganglia contain a small number of 5-hydroxytryptamine (5-HT)-positive neurons (approx. 14 nerve cells per SO preparation) but the serotonergic nerve fibres are densely distributed throughout the sphincter ganglionated plexus. The 5-HT-positive varicose nerve terminals are often found in a close association with the ganglionic neurons. They are also distributed in the SO circular muscle. 5-hydroxytryptamine has been revealed to elicit a variety of electrophysiological responses in guinea-pig SO neurons (Hillsley and Mawe 1998b). They include fast and prolonged depolarization mediated by 5-HT\textsubscript{3} and 5-HT\textsubscript{1P} receptors, respectively, and an indirect effect that involves the stimulation of cholinergic interneurons (supplying the SO neurons) via 5-HT\textsubscript{3} receptor.

Mawe and Kennedy (1998, 1999) have performed two experiments in the guinea pig using retrograde tracing method combined with immunohistochemistry to investigate the distribution and chemical coding of intrinsic neurons supplying the SO. In the first study (Kennedy and Mawe 1998) they have applied DiI into the SO. This investigation revealed 116 neurons (DiI-positive) in the duodenal myenteric plexus projecting to the sphincter. The vast majority of them (approx. 92%) were located within 5 mm of the injection site. Furthermore, it was found that most of MDP/SO-projecting myenteric neurons were ChAT-positive but did not contain immunoreactivities for NOS or calretinin. This is an interesting finding because Clerc et al. (1998a,b) have established that the vast majority of guinea pig duodenal myenteric neurons are immunoreactive for either ChAT or NOS but not both enzymes, thus the neurons observed by Kennedy and Mawe (1998) belonged exclusively to the supopulation of the cholinergic neurons. Moreover, approx. 20% of the DiI-positive neurons exhibited immunoreactivity for calbindin (Kennedy and Mawe 1998), a marker of intrinsic sensory neurons in the guinea pig enteric nervous system. Kennedy and Mawe (1998) have also revealed that duodenum-SO projecting neurons are depolarized by cholecystokinin (CCK). Because calbindin-immunoreactive myenteric neurons project to the mucosa (Furness et al. 1990) and postprandial release of CCK also occurs in the mucosa, the authors have stated that the role of the intrinsic sensory neurons projecting to the SO may be to signal the postprandial release of CCK, thus providing the information to decrease SO resistance and facilitate the flow of bile into the duodenum.

In another experiment Mawe and Kennedy (1999) have administered DiI into the duodenal wall close (3 mm) to the MDP. They found approx. 46 neurons in the SO labeled with the tracer. Many DiI-positive axons could be traced from the labeled nerve cell bodies in the sphincter into the myenteric plexus of the duodenum. This study has also revealed that approx. 26% or 20% of SO neurons receiving fast synaptic input from the duodenum or duodenal mucosa, respectively, are nitrergic in nature (NOS-positive). The authors have concluded that bidirectional neuronal communication occurs between the duodenum and the SO, and that duodenal neurons provide excitatory fast synaptic input to SO neurons through a reflex that can be activated at the duodenal mucosa.
In light of the above it seems worth mentioning that in the guinea pig the chemical coding of SO neurons resembles not only that of nerve cells in the myenteric plexus of the duodenum but also of the stomach. In this species, two distinct populations of neurons in the gastric myenteric ganglia can be distinguished including those (approximately 63%) containing ChAT and those (33%) expressing NOS (for review, see Schemann et al. 2001). This specific neurochemical organization of the gastric enteric nervous system reflects clearly polarised motor pathway projections consisting of ascending excitatory cholinergic and descending inhibitory nitrergic populations.

The presence of ganglia closely associated with the SO smooth muscle in the monkey (Macaca fascicularis) has been revealed by Melander et al. (1991). These authors have found that in the monkey, the CBD and the PD merge together within the same muscular tube just before entering the duodenal wall. However, the microscopy analysis has disclosed that both ducts are separate components within this common structure entering the intestinal wall, with individual circular smooth muscle layers. Outside these two structures a common circular sphincter smooth muscle surrounds the CBD and PD. The clusters of nerve cells (referred by the authors to us “local ganglia”) were distributed in a close association with the sphincter smooth muscle outside the gut wall. Unfortunately, the information on the chemical coding of these neurons contained in the paper of Melander et al. (1991) is very limited. The authors found that the SO ganglia were heavily supplied by ENK- or tyrosine hydroxylase (TH)-positive nerve fibres. These nerve terminals abundantly supplied also the sphincter smooth muscle as well as the sphincters of the CBD and PD. The sphincters were also intensely innervated by NPY-positive axons, however, no NPY-IR innervation of the local ganglion cells could be detected. The biologically active substances investigated by Melander et al. (1991) were found to be expressed in the nerve fibres associated with the SO ganglia (preganglionic fibres or collaterals of afferent cells (preganglionic fibres or collaterals of afferent neurones)). Whether these nerve terminals within the MDP/SO in different mammalian species, the information they provide, in the context of the present contribution, should be considered as incomplete and none of them presents data based on the neuronal tracing method. Furthermore, it is also unclear exactly what tissues are targeted by these nerve terminals within the MDP. Whether they represent fibres supplying the local ganglionic nerve cells (preganglionic fibres or collaterals of afferent fibres associated with ganglionic neurons) or whether they represent axons innervating the SO musculature. Nevertheless, below we briefly outline the core body of knowledge (in addition to that already presented in this article) on the expression of biologically active substances in nerve terminals associated with the mammalian SO and their physiological functions.

Application of histochemical methods has revealed the occurrence of adrenergic and cholinergic nerve fibres associated with the SO in the cat and dog (Kyösoila and Rechardt 1973, Kyösoila 1974), and guinea pig (Cai and Gabella 1983a).

The VIP-immunoreactive nerve terminals have been found in the cat (Dahlstrand et al. 1990a, Feher et al. 1995) and this peptide has been revealed to

Unauthenticated
cause a dose-dependent relaxation of the feline SO (Dahlstrand et al. 1989a,b). As already mentioned, CCK should be considered as one of the most important factors of the SO function, and VIP has been suggested to act as a mediator of CCK action in cats (Dahlstrand et al. 1990b).

Neuropeptide Y-positive nerve fibres have been observed in the biliary tract of the guinea pig (Allen et al. 1984) and cat (Feher et al. 1995), and this peptide appears to have an excitatory effect on the prairie dog SO (Lillemoe et al. 1988).

As already mentioned, SP and CGRP are expressed by many nerve terminals supplying the MDP/SO in the guinea pig (Wells et al. 1995) and pig (Sand et al. 1993). Large number of SP-positive nerve fibres associated with the SO have been also found in the cat (Feher et al. 1995). Substance P and CGRP have appeared to have an excitatory effect on the opossum, guinea pig, canine and porcine SO (Guo et al. 1989, Parodi et al. 1989, 1990, Rasmussen et al. 1997, Manning and Mawe 2001), and the effect of CGRP on the sphincter function involves cholinergic but not cholecystokininergic mechanisms (Rasmussen et al. 1997).

Galanin-positive nerve fibres supplying the SO have been observed in the pig (Harling et al. 1991) and Australian brush-tailed possum (Baker et al. 1996). It should be mentioned that Harling et al. (1991) have found not only the nerve fibres but also GAL-positive neurons in the porcine sphincter (however, no detailed data on their distribution have been provided). This peptide has been found to inhibit the porcine SO contractility (Harling et al. 1991). In the possum, GAL has been revealed to selectively stimulate longitudinally oriented sphincter smooth muscle via a direct mechanism, which results in a moderate reduction in transmepithelial flow (Baker et al. 1996).

Nerve terminals expressing SOM-immunoreactivity associated with the SO have been observed in the guinea pig (Cai et al. 1983b) and cat (Feher et al. 1995). Exogenous SOM has been revealed to have an inhibitory effect on the SO in the rabbit (Adami et al. 1986) and in the prairie dog (Ahrendt et al. 1990). In humans (Wu et al. 2005) and dogs (Wang et al. 2000) SOM and its analogues have been found to affect SO motility in a dose-dependent manner; small doses of the agents stimulate while the large doses inhibit the motility.

Bombesin-immunoreactive SO associated nerve fibres have been found in the guinea pig (Cai et al. 1983b). The effect of this peptide on the SO function is vague. In the dog, administration of BOM has been found to result in the sphincter relaxation and gallbladder contraction; however, these effects were mediated by endogenous CCK rather than by a direct action (Sievert et al. 1988). On the other hand, in rabbits, administration of BOM increased the spikes activity of the SO.

The results of the above mentioned physiological studies undertaken to clarify the action of biologically active substances on the SO should be interpreted cautiously. As we know these structures are innervated (among others) by the local intrinsic neurons (frequently distributed among SO smooth muscle layers) and it is unclear whether the substances investigated affect the SO muscles directly or whether they can influence the sphincter via the local nerve cells.

Extrinsic innervation of the major duodenal papilla and sphincter of Oddi (Fig. 1)

It should be emphasized that application of the retrograde tracing method is commonly considered to be one of the most advanced and precise approaches in localising specific neuronal populations supplying any particular organ under study (Kaleczyc 1998). However, the information on the anatomical organization of the extrinsic innervation of the MDP/SO based on tracing experiments is much more limited than that on the intrinsic nerve supply.

Efferent innervation

To date, investigations dealing with sources of the extrinsic innervation of the SO with the use of the retrograde tracer FB have been carried out only in the Australian possum by Padbury et al. (1993b). These authors have found the retrogradely labeled nerve cells in the coeliac-anterior mesenteric ganglion complex (CAMG). About half of the neurons displayed immunoreactivity to GAL. However, no information has been provided on the contribution of sympathetic chain (paravertebral) ganglia to the SO nerve supply. Therefore, it is difficult to appraise whether CAMG is the only source of sympathetic (presumably adrenergic) nerve fibres supplying the possum sphincter.

Furthermore, as already mentioned, the SO in the Australian possum possesses well developed local intramural nerve plexuses comprising ganglia. It can be assumed that the preganglionic input to these ganglia is provided by the vagus nerve. However, no information based on tracing experiments is available on the contribution of the dorsal motor nucleus of the vagus nerve (DMV) to this preganglionic nerve supply. Nevertheless, there are some data concerning the influence of the vagus nerve on the SO motility in a variety of species. Fukurawa and Okada (1992) have revealed that stimulation of the canine DMV affects the...
SO motility, but the effects depended on which portion of the nucleus was stimulated. Stimulation at the rostral portion elicited only excitatory effects. Stimulation at the middle portion induced excitatory or combined, excitatory and inhibitory effects. Stimulation at the caudal portion elicited the three different effects. Moreover, some authors have investigated the effect of vagotomy on the SO motility in different species. However, the results obtained are inconclusive. Liedberg and Halabi (1970) have observed no changes in SO function after vagotomy in dogs and cats. Pitt et al. (1982) have found that vagotomy increased the SO phasic wave activity in prairie dogs. On the other hand, Takahashi et al. (1988) have revealed that this surgery does not affect the fasting standard of the SO contractile activity in the opossum, but alters the normal postprandial activity of the sphincter by eliminating its spike-bursts (contractions) pace. Nabae et al. (2002) reported that SO phasic contractions in dogs were significantly increased before feeding, but they were decreased definitively after meal. Truncal vagotomy abolished both responses, suggesting that the vagal tone, which is stimulated by food, may affect the SO functions. Moreover, Ohtsuka et al. (2002) denervated the canine biliary sphincter and found that temporary inhibitory effect of feeding was absent. They have concluded that nerves supplying the sphincter play an inhibitory role in delivering bile into the duodenum after feeding.

It is also important to mention that the population of ganglionic autonomic neurons consists of not only adrenergic and cholinergic nerve cells but also comprises a small subpopulation of non-adrenergic, non-cholinergic (NANC) neurons. Behar and Biancani (1980) have suggested that the feline SO receives NANC nerve supply. They observed that the relaxation of the SO caused by CCK was not antagonized by adrenergic or cholinergic blockade. NANC innervation appears to be inhibitory and largely mediated by NO as the main neurotransmitter (Baker et al. 1993). As already stated, NO is a well recognized transmitter in neurons supplying the SO in several species (Woods et al. 2005) including the guinea pig.

Afferent innervation

Investigations involving administration of retrograde tracers such as FB and DiI to identify sources of the afferent neural input to the SO and gallbladder have been performed by Padbury et al. (1993b) in the Australian possum. After injection of FB into the sphincter, they have found labeled nerve cell bodies in the bilateral nodose ganglia of the vagus nerve, and in dorsal root ganglia (Th8-Th10). However, no information has been provided on the number of the labeled neurons in particular ganglia, thus nothing is known about their percentage participation to the possum SO nerve supply. Padbury et al. (1993b) have obtained similar results concerning the gall bladder which in turn correspond to findings obtained in another study dealing with the afferent innervation of this structure performed in the guinea pig by Mawe and Gershon (1989).

It should be stated that there is also some indirect evidence suggesting the contribution of sensory ganglia to the innervation of the SO. As mentioned before, the sphincter is well supplied by nerve fibres exhibiting immunoreactivities to SP and/or CGRP, thus neuropeptides commonly considered as markers of the peripheral efferent nerve supply.

Conclusion

Although quite large body of diverse (morphological and physiological) data on the innervation of the mammalian SO has been accumulated so far, there is still a paucity of some fundamental information dealing with this issue. The majority of the comprehensive morphophysiological studies have focused on the organization of the intrinsic nerve supply. These investigations have revealed the existence of a local complex network of intramural duodenal neurons associated with the SO. Some of them are distributed within the sphincter (local ganglionic neurons). Moreover, these local nerve cells not only supply the sphincter muscle but some of them project outside to supply neighboring duodenal structures. Thus, it can be assumed that bidirectional neuronal communication occurs between the duodenum and the SO which seems to be essential for the flow of bile into the intestine.

In contrast to the relatively large amount of the knowledge (in some species: the guinea pig, Australian brush tailed possum) on the intrinsic innervation of the SO, the detailed information on the arrangement of the extrinsic innervation is very limited and fragmentary. This shortage of data deals mainly with the information about the localization, number and chemical coding of the preganglionic, sympathetic and sensory neurons based on neuronal tracing studies. Therefore, the contribution of sympathetic chain ganglia to the sphincter innervation as well as characterization of the preganglionic neurons (also those supplying the intrinsic local ganglia closely associated with the SO) and the neurons in ganglia of the vagus nerve and dorsal root ganglia still remain to be satisfactorily documented.
References


Mawe GM, Gershon MD (1989) Structure, afferent innerva-
tion, and transmitter content of ganglia of the guinea pig
gallbladder: relationship to the enteric nervous system.

Mawe GM, Kennedy AL (1999) Duodenal neurons provide
nicotinic fast synaptic input to sphincter of Oddi neurons

Mawe GM, Talmage EK, Cornbrooks EB, Gokin AP, Zhang
L, Jennings LJ (1997) Innervation of the gallbladder:
structure, neurochemical coding, and physiological
properties of guinea pig gallbladder ganglia. Microsc Res

Melander T, Millbourn E, Goldstein M (1991) Distribution of
opioidergic, sympathetic and neuropeptide Y-positive
nerves in the sphincter of Oddi and biliary tree of the
monkey, Macaca fascicularis. Cell Tissue Res 266:
597-604.

Nabae T, Yokohata K, Otsuka T, Inoue K, Yamaguchi K,
Vagotomy on Sphincter of Oddi Cyclic Motility in Con-

Ohtsuka T, Yokohata K, Inoue K, Nabae T, Takahata S,
sphincter motility after neural isolation of the pancre-
ato-duodenal region in conscious dogs. Surgery
131: 139-148.

Padbury RT, Baker RA, Messenger JP, Touloli J, Furness JB
(1993a) Structure and innervation of the extrahepatic bi-
ary system in the Australian possum, Trichosurus vul-
pecula. HPB Surg 7: 125-139 discussion 139-40.

(1993b) Projections of nerve cells from the duodenum to
the sphincter of Oddi and gallbladder of the Australian

Parodi JE, Cho N, Zenilman ME, Barateau JA, Soper NJ,
Becker JM (1999a) Substance P stimulates the opossum

Parodi JE, Zenilman ME, Becker JM (1989) Character-
ization of substance P effects on sphincter of Oddi my-

Pautletzki JG, Sharkey KA, Davison JS, Bomzon A, Shaffer
EA (1993) Involvement of L-arginine-nitric oxide path-
ways in neural relaxation of the sphincter of Oddi. Eur J
Pharmacol 232: 263-270.

Altered sphincter of Oddi phasic activity following trun-

Purvis NS, Mirjalili SA, Stringer MD (2013) The mucosal
folds at the pancreatico-biliary junction. PMID: 23645171

icitonin gene-related peptide (CGRP), a potent regulator

sphincter of Oddi, bile duct, gallbladder, and duodenum.

293-300.


Sievert CE Jr, Potter TJ, Levine AS, Morley JE, Silves SE,
Vennes JA (1988) Effect of bombesin and gastrin-releasing
peptide on canine sphincter of Oddi. Am J Physiol
254: G361-365.

Simula ME, Brookes SJ, Meedeniya AC, Touloli J, Saccone
GT (2001) Distribution of nitric oxide synthase and
vasoactive intestinal polypeptide immunoreactivity in the
sphincter of Oddi and duodenum of the possum. Cell

Simula ME, Harvey JR, Costi D, Baker RA, Touloli J, Sac-
ccone GT (1997) In vitro characterisation of intramural
neural pathways between the duodenum and the sphin-
ter of Oddi of the brush-tailed possum. J Auton Nerv
Syst 63: 77-84.

Effect of vagotomy on biliary-tract motor activity in the

Identification of the cholinergic neurons in guinea-pig

Touloli J, Baker RA (1991) Innervation of the sphincter of
Oddi: physiology and considerations of pharmacological
intervention in biliary dyskinesia. Pharmacol Ther
49: 269-281.

its analogue on canine sphincter of Oddi motility. Zhon-
ghua Yi-Xue Za Zhi 80: 621-623.

Wells D, Mawe G (1993) Physiological and morphological
properties of neurons in sphincter of Oddi region of the
pig. Am J Physiol 265: G258-G269

identification of neurons in ganglia of the guinea pig

Woods CM, Mawe GM, Touloli J, Saccone GT (2005) The
sphincter of Oddi: understanding its control and func-

of the sphincter of Oddi. Curr Gastroenterol Rep
9: 165-170.

Wu SD, Zhang ZH, Kong J, Li YJ, Jin JZ, Wang W, Li DY,
Wang MF (2005) Effects of somatostatin analogues on
human sphincter of Oddi pressure. Hepatobiliary Pancre-
at Dis Int 4: 302-305.