

PHARMACOLOGICAL CHARACTERIZATION OF AN
ESOPHAGO-CARDIOVASCULAR REFLEX IN THE RAT

CENTRE FOR NEWFOUNDLAND STUDIES

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DONGYUAN YAO



Pharmacological Characterization of An Esophago-Cardiovascular Reflex in the Rat

by

Dongyuan Yao

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ABSTRACT

The distension of hollow viscera is known to evoke a range of autonomic visceromotor and behavioral responses subsumed under the term "pseudoaffective" reflex. Although some physiological properties of "pseudoaffective" reflex responses to esophageal distension have been previously described in the rat, only limited pharmacological data are currently available. The present investigation focused on an esophageal distension-evoked pressor/cardioaccelerator response in the urethane-anaesthetized rat. Our primary objectives were: i) to determine whether relevant sensory input from the esophagus to the CNS is conveyed by vagal or spinal afferents; and ii) to study the effects of antinociceptive agents on this reflex, and their sites of actions. Experiments were carried out on male, Sprague-Dawley rats (300-450 g), previously prepared for intrathecal (i.t.) injection at predetermined spinal segments, and anaesthetized with urethane (0.8 g + 20 mg/kg/h i.v.). Esophageal distension evoked a reproducible increase in arterial blood pressure and heart rate. Both components increased linearly with the log of inflation pressure (25-150 mmHg). Lower, being more effective than upper esophageal distension (100 mmHg for 20 sec), was insensitive to i.t. thoracic morphine (4-16 µg). However, i.v. morphine (1.0-4.0 mg/kg) produced a dose-dependent inhibition of the evoked responses. This esophago-cardiovascular reflex was: attenuated by unilateral, and abolished by bilateral cervical vagotomy; and inhibited by the i.t. thoracic, but not by i.t. lumbar or i.v. injection of dexmedetomidine (DX, 0.05-0.5 µg). The evoked response was inhibited by neonatal capsaicin, and by the topical administration of DX or morphine to the solitary complex (NTS). The pressor response persisted after i.v. pancuronium, scopolamine, and methscopolamine. The data indicate that: 1) the sensory information of this esophago-cardiovascular reflex is conveyed to the CNS via vagal afferents; 2) the efferent limb of this reflex is comprised of sympathetic cardioaccelerator- and vasoconstrictor-preganglionic pathways in the IML of the thoracic spinal cord; and 3) the internuncial connections are made up of bulbo-spinal pathways probably originating in the RVLM which in turn, receive input from the NTS.

Key words: esophageal distension; morphine; dexmedetomidine; intrathecal; solitary complex; spinal; vagal; pseudoaffective reflex; esophageal pain; rat

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LIST OF ABBREVIATIONS AND SYMBOLS

α	alpha, a Greek letter
β	beta, a Greek letter
γ	gamma, a Greek letter
μ	mu, a Greek letter used to indicated micro in metric units, also a subtype of opioid receptor
δ	delta, a Greek letter used to indicated micro in metric units, also a subtype of opioid receptor
$^{\circ}\text{C}$	degrees Celsius
=	equals
>	greater than
<	less than
μA	microampere(s), 10^{-6} amperes, unit of electric current
μg	microgram(s), 10^{-6} grams, unit of mass
μl	microliter(s), 10^{-6} litres, unit of volume
μm	micrometer(s), 10^{-6} meters, unit of distance
μM	micromolar, 10^{-6} moles per litre, unit of concentration
μmol	micromole(s), 10^{-6} moles, unit of molecules
nmol	nanomole(s), 10^{-9} moles, unit of molecules
+	plus, in combination with
A δ	A-delta, a class of primary afferent neurons
ANOVA	analysis of variance
bpm	beats per minute
C	a class of primary afferent neurons
C1	cervical vertebra number one
CCK	cholecystokinin
C.I.	confidence of interval (about the mean)
CGRP	calcitonin gene-related peptide
CNS	central nervous system
Co.	company, corporation
DRG	dorsal root ganglion
DX	dexmedetomidine
e.g.	exempli gratia, (for example)
ED ₅₀	effective dose for 50 percent response
EKG	electrocardiogram
<i>et al.</i>	<i>et alia</i> (and others)
EMG	electromyography
Fig.	figure
g	gram
h	hour(s)

HCl	hydrochloric acid
Hg	elemental symbol for mercury
H ₂ O ₂	hydrogen peroxide
HR	heart rate
HTMN	high-threshold mechanonociceptor(s)
Hz	hertz, S ⁻¹ , reciprocal seconds, unit of frequency
I	Roman numeral one, used to denote spinal lamina
i.e.	<i>id est</i> , that
IGLE	intraganglionic laminar nerve ending
i.p.	intraperitoneal(ly)
i.t.	intrathecal(ly)
i.v.	intravenous(ly)
II	Roman numeral two, used to denote spinal lamina
III	Roman numeral three, used to denote spinal lamina
IV	Roman numeral four, used to denote spinal lamina
IR	immunoreactivity
IML	intermediolateral cell column
Inc.	incorporated
<i>in vitro</i>	in glassware
<i>in vivo</i>	in the living body
kg	kilogram(s), 10 ³ grams
Kpa	kilopascal(s), unit of pressure
L1	lumbar vertebra number one
LES	lower esophageal sphincter
L-ENK	leucine enkephalin
log	logarithm (base 10)
m	meter(s)
MAP	mean arterial blood pressure
mg	milligram(s), 10 ⁻³ gram
MK-212	6-chloro-2-(1-piperazinyl)-pyrazine
ml	milliliter(s), 10 ⁻³ liter
mm	millimeter(s), unit of distance
mm ²	millimeter(s) squared, unit of area
M	molar (moles/liter)
N	number of determinations
nmol	nanomole(s), 10 ⁻⁹ moles, number of molecules
NTS	nucleus of the solitary tract
NTSc	central subnucleus of the solitary tract
NX	naloxone
PAP	peroxidase-antiperoxidase
P.E.	polyethylene tubing
PE-10	size 10 polyethylene tubing (diameter=0.61 mm)

p	probability of error
pH	negative log of the hydrogen ion concentration
RVLM	rostromedial lateral medulla
sec	second(s)
s.c.	subcutaneous(ly)
SEM	standard error of the mean
SP	substance P
SST	somatostatin
T1	thoracic vertebra number one
U	biological units
VIP	vasoactive intestinal peptide
WDRMN	wide-dynamic range mechanonociceptor(s)
WY27127	Wyeth 27127 (disulphonamido-benzoquinoline)
w/v	weight per volume
V	Roman numeral five, used to denote spinal lamina
VI	Roman numeral six, used to denote spinal lamina
X	times (multiplied by), also Roman numeral ten

INTRODUCTION

Statement of the Research Problem

Recurrent chest pain is an important and potentially serious clinical symptom because of its association with cardiac disease. However, not all recurrent chest pain is of cardiac origin. Of the approximately 600,000 new patients with anginal symptoms who undergo cardiac catheterization in the United States each year, 30% have normal coronary arteries. Of those patients with normal coronary circulation who report chest pain, approximately 50% have a demonstrable esophageal abnormality (Richter *et al.*, 1989).

Esophageal motility disorders, characterized by high amplitude, long lasting, peristaltic contractions, are observed in approximately 28% of patients with noncardiac chest pain (Richter *et al.*, 1989). Chest pain in humans also arises from spontaneous contractions of the esophagus against a non-compressible object in the lumen (Payne and Poulton, 1927; Nixon and Koch, 1989), and from distension of the esophagus itself (DeVault and Castell, 1992). Depending on the degree of distension, patients have described esophageal chest pain as 'a severe dull ache' and 'something sharp' to 'a continuous burning and gripping pain' (Payne and Poulton 1927; Payne and Poulton, 1928; Pollard and Bloomfield, 1931a). Prolonged distension also causes cutaneous hyperaesthesia in the skin above the sternum.

Esophageal pain is often referred from the area of the suprasternal notch to xiphoid process and sometimes to the costal angle, with radiation through to the

angle of the scapula. As a result, esophageal pain can mimic the pain of myocardial ischemia making it very important for the physician to first properly diagnose the cause of the pain. At the present time, it is very difficult to distinguish cardiac from esophageal chest pain until after extensive testing has been completed. This delay in diagnosis can result in unnecessary stress to both patient and physician, and the need for extensive cardiac tests imposes significant costs to the health care system. It is estimated that more than 315 million dollars are spent each year in the United States to diagnose patients with esophageal pain (Richter *et al.*, 1989).

Underlying this clinical problem is a lack of information about visceral pain in general, and more specifically, the lack of information about the mechanism(s) of and the neural pathways mediating esophageal pain. Although the extrinsic innervation of the esophagus is well known, the role of spinal and/or vagal afferent fibers in esophageal pain is unclear. Using graded esophageal distension as the visceral stimulus, the purpose of this study was: 1) to determine the relevant sensory pathway(s) that trigger reflex "pseudoaffective" cardiovascular responses in urethane-anesthetized rats; and 2) to investigate the pharmacological properties of this esophago-cardiovascular reflex.

General Features of Visceral Pain

Pain is generally classified as either superficial or deep depending on its location in the body. Superficial pain arises from noxious stimulation to cutaneous

structures and the mucous membranes of body orifices. Deep pain originates in muscle, fascia, bone, joints, vascular structures and the viscera. Visceral pain is a special kind of deep pain. It is evoked by stretching, twisting, or contraction of an organ and is the most common type of pain treated by physicians. Unlike superficial pain, visceral pain is dull in nature, poorly localized, and generally evokes an intense emotional response. It is often referred to cutaneous but otherwise normal sites on the body, and has a propensity to trigger tonic muscle contractions as well as autonomic responses (collectively called the pseudoaffective responses). Whereas deep pain from musculoskeletal and articular tissue can generally be diagnosed using criteria such as the rate of onset of pain, the location of referred sensations and the presence of aggravating factors, visceral pain is very difficult to diagnose on the basis of the quality, location and intensity of sensation alone. Cardiac pain is particularly difficult to distinguish from that originating in the esophagus, liver, appendix and gallbladder (see Ness and Gebhart, 1990 for review). Clinical tests and imaging techniques are currently required for this purpose.

Sensory Innervation of the Esophagus

The innervation of the esophagus is supplied bilaterally by the vagus nerve and by spinal nerves that extend from the cervical to thoracic segments of the spinal cord (Fig. 1). Each of these two general pathways are discussed in detail below.

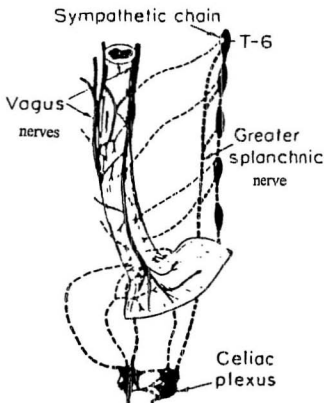


Figure 1. Neural innervation of the human esophagus. (Source: Ellis, 1981)

Vagal Afferent Fibers

Vagal afferent fibers arise from cell bodies in the superior (jugular) and inferior (nodose) ganglia of the vagus. The central axonal processes project via the tractus solitarius to the nucleus of the tractus solitarius tractus, while the peripheral axons primarily terminate between the longitudinal and circular muscle layers, in and around the myenteric ganglia, with only scattered fibers innervating the submucosal and mucosal layers (Neuhuber, 1987). The ratio of afferent: efferent fibers for individual branches of the vagus is not clear, but about 80-90% of the fibers in the abdominal vagi are afferent, unmyelinated C fibers in non-ruminant animals (Andrews, 1986). The remainder are small myelinated and large myelinated fibers (Woodbury and Woodbury, 1990).

Different types of vagal afferent fibers subserve different sensory functions in the esophagus. Recent studies (Sengupta *et al.*, 1989) have shown that tension-sensitive (mechanoreceptor) afferent fibers in the opossum are of the C- or A δ -type. Mechano and chemoreceptors in the mucosa are present on B-fibers (Harding and Titchen, 1975). Thermoreceptors in the esophageal mucosa of the cat are located on small diameter myelinated fibers in the vagus nerve (El Ouazzani and Mei, 1982).

Mechanoreceptive afferents from the esophagus have a low level of spontaneous activity and exhibit an increased discharge to either moderate distension or muscle contraction (Andrews, 1986). In the opossum, the threshold distension pressure (applied 2-7 cm above lower esophageal sphincter) required to

evoke activity in these vagal afferent fibers is <10 mmHg. Maximal discharge is achieved at a pressure of 56 mmHg for discrete distension (70 mmHg for stepwise distension). Three general phases of response to esophageal distension are exhibited by these fibers. The first phase is characterized by a rapid increase in firing during the onset of the distension. This is followed by a lower discharge rate (second phase) that remains above the level of resting activity for as long as the esophageal distension pressure is maintained. Before returning to normal resting activity, the frequency discharge falls below the resting level and some fibers even stop firing for 2-4 seconds after the distension pressure is discontinued (third phase) (Sengupta *et al.*, 1989).

The data indicate that these vagal afferent fibers, which occur in series with the circular or longitudinal muscle layers of the esophagus: a) are slowly adapting; b) have a low threshold of activation (<10 mmHg) and a maximal response within the physiological range of esophageal distension pressure; and c) are maximally activated by normal peristaltic contractions (Sengupta *et al.*, 1989). Recent studies (e.g. Randich *et al.*, 1990, 1991, Møller *et al.*, 1991, Ren *et al.*, 1991) have confirmed the role of vagal afferents in nociception. For example, Randich *et al.* (1991) showed that the antinociceptive effects of low doses of i.v. morphine (0.1 or 0.5 mg/kg) involve activation of vagal afferents. Bilateral cervical vagotomy significantly attenuated the antinociception produced by i.v. morphine and the degree of attenuation was inversely related to drug dose. As reviewed by Randich

and Gebhart (1992), electrical stimulation of right or left cervical vagal nerve (afferent) either facilitates tail flick reflex at lesser intensities (typically 2.5-20 μ A, 20Hz, 2 ms) or inhibits the tail flick reflex at greater intensities ($>30 \mu$ A) in an intensity-dependent manner in the rat. These data suggest that the effects of peripheral stimuli on nociception depends on integrity of vagal nerves. The importance of vagal afferents in esophageal nociception is not fully understood.

Spinal Afferent Fibers

While all regions of the esophagus receive both vagal and spinal afferent innervation, the cervical and upper thoracic parts of the esophagus are predominantly supplied by vagal afferents. The lower part of thoracic esophagus and the abdominal esophagus are primarily innervated by sympathetic afferents. The abdominal portion of the esophagus is also innervated by the phrenic nerve (Pope and Bonica, 1990). In the cat, spinal nerves innervating the lower esophageal sphincter extend from spinal segments T1 to L3, with a peak distribution in T1-T12. The innervation of esophageal smooth muscle extends from spinal segments C4 to L3, with a peak distribution in T1-T12. Esophageal striated muscle is supplied by nerves from spinal segments C1 to T8, with peak distributions in C4-C6 and T2-T7 (Fig. 2) (Collman *et al.*, 1992). In the dog, the cervical esophagus is innervated by spinal afferents whose cell bodies are located in C1-T9 dorsal root ganglia; the peak distribution is located in C1-C6 and T3-T4 (Khurana and Petras, 1991). In general,

spinal afferents to esophagus, which are few in number, arise from lower cervical and upper thoracic dorsal root ganglia (see Cunningham and Sawchenko, 1990 for review).

The sympathetic fibers that supply the esophagus originate in the intermediolateral (IML) column of the spinal cord. In humans, the cell bodies of preganglionic sympathetic neurons are located in the spinal segments T2-T8 (Pope and Bonica, 1990). Their axons pass from the spinal cord through the ventral nerve roots and continue via the corresponding spinal nerves to reach the paravertebral sympathetic chain. Primary afferent fibers from the esophagus project to spinal cord mostly in the splanchnic and sympathetic nerves. After passing through the paravertebral sympathetic chain and the rami communicantes, they join the spinal nerves before entering the dorsal root ganglia.

The central terminations of these spinal afferent fibers have not been studied in detail but Euchner-Wamser *et al.* (1993) showed that neurons in spinal segments T2-T4, which are activated by distension in the middle region of the esophagus, are distributed throughout lamina I-VI, with a peak distribution in the deeper laminae.

Esophageal Receptors and Pain

As described above, sensory information is conveyed from the esophagus to the CNS by vagal and spinal afferent fibers. Vagal afferents in the submucosal and mucosal layer may act as mechano-, thermo-, or chemoreceptors (Cunningham

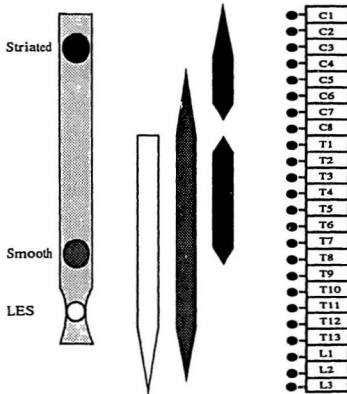


Figure 2. Distribution of retrogradely labelled cells in dorsal root ganglia (DRG) (right) after injection of fast blue into the cervical striated muscle (black blocks) and thoracic smooth muscle (gray block) of the esophagus. The rectangular portion of each block denotes the DRGs that were labelled in four of four cats; the pointed part of each block signifies that not all animals had labelling at this level. LES = lower esophageal sphincter (From Collman *et al.*, 1992)

and Sawchenko, 1990; Harding and Titchen, 1975). These mechanoreceptors and chemoreceptors are ideally suited for detecting gastric contents that are refluxed into the esophagus and which normally yield distension pressures too low to activate muscle receptors. These mucosal receptors may play an important role in vagally mediated reflexes such as secondary peristalsis and the clearing of refluxed gastric contents (Christensen, 1984, Andrews, 1986).

Thermoreceptors are also found on the peripheral terminals of vagal afferents in the esophageal mucosa. Three types of slowly-adapting thermoreceptors have been detected in the cat: 1) warm receptors that discharge between 39 to 50°C; 2) cold receptors that discharge between 10 and 35°C; and 3) mixed receptors, responding to both warm and cold temperatures (El Ouazzani *et al.*, 1982). These receptors were silent at normal body temperature. The receptor discharge was slow (1-20 impulses/s), lasted for 1-20 seconds, and exhibited a discharge frequency that was generally related to the stimulus temperature. These receptors are important in the detection and transduction of temperature changes in the lumen of the esophagus.

Slowly adapting mechanoreceptors, located in the muscular and "serosal" layers of the esophagus, are connected to both vagal and spinal afferent fibers (Clerc and Mei, 1983a, 1983b, Satchell, 1984). Mechanoreceptors on spinal afferent fibers exist mainly in the muscle layers of the esophagus. They are less frequent in the "serosal" layer and absent in the mucosa (Lynn, 1992). There is also

evidence that slowly adapting esophageal mechanoreceptors can be functionally subdivided (Satchell, 1984), suggesting that the characteristics of mechanoreceptors on vagal afferent fibers are different from those on spinal afferents.

These characteristics have been studied in the opossum (Sengupta *et al.*, 1983, 1990, 1992) and in the dog (Satchell, 1984). Distension-sensitive vagal afferents have a low threshold of activation (average threshold pressure = 10 mmHg), a narrow stimulus-response function in which the unit discharge rate increases with increasing distension pressure, and reaches a maximum discharge rate at distension pressures within the normal physiological range (< 56 mmHg) (Sengupta *et al.*, 1989). In humans and experimental animals, esophageal distension pressures greater than 60 mmHg are usually required to elicit a nociceptive response (Lynn, 1992). The functional importance of the vagal mechanoreceptors is not fully understood. However, it is likely that pressure information on tension in circular and longitudinal muscle layers of the smooth muscle portion of the esophagus is sent to the central nervous system by vagal nerves during esophageal distension and esophageal peristalsis (Sengupta *et al.*, 1989).

In contrast, the mechanoreceptors on spinal afferent fibers in the opossum have been shown to be primarily nociceptive (Sengupta *et al.*, 1990, Lynn 1992). They appear to exist "in series" with the longitudinal muscle in the esophagus and can be divided into two types: wide-dynamic range mechanonociceptors (WDRMN)

and high-threshold mechanonociceptors (HTMN). The WDRMN is characterized by a low threshold of stimulation and a linear stimulus response function extending from innocuous to noxious intensities. It can distinguish between physiological and nociceptive stimuli, and may subserve a role in both physiological reflexes and nociception (Sengupta *et al.*, 1990). The HTMN responds only to high-intensity mechanical stimuli in the noxious range and may serve a specific nociceptive role (Sengupta *et al.*, 1990). The discharge rate of mechanoreceptors located on spinal afferent fibers, activated by esophageal distension, does not reach plateau at pressures up to 120 mm Hg.

Information about the structure of esophageal mechanoreceptors is limited. The spindles found in somatic striated muscle are mechanoreceptive sensors and it has been suggested that they serve a similar function in the striated muscle of the esophagus (Christensen, 1984). In the dog, these spindles are found in the muscularis propria and intermuscular space in the lower one-third of the esophagus (Asaad *et al.*, 1983). However, they are not present in smooth muscle, suggesting that at least one other type of mechanoreceptor exists in the esophagus. It is possible that nerve cell bodies themselves are mechanoreceptive or that special structures exist within the nervous elements of smooth muscle (Christensen, 1984). Studies by Neuhuber (1987) have shown that myenteric vagal sensory terminals are identical with the intraganglionic laminar nerve endings or IGLEs described by Rodrigo *et al.* (1982), which may be these special mechanoreceptive structures.

Although many details remain to be determined about the sensory innervation of the esophagus, it is clear that primary afferent fibers in both the vagus and spinal nerves subserve a sensory role in the esophagus. Their peripheral terminals contain mechanoreceptors that are activated by distension of the esophagus, and are thus theoretically capable of conveying visceral nociceptive information from the esophagus to the CNS. The actual role played by each of these nerve routes in esophageal pain is unknown and is a major focus of the present study.

Pseudoaffective Reflexes to Pain

Besides evoking the sensation of "pain", visceral noxious stimuli elicit a characteristic pattern of reflex responses that were initially characterized by Sherrington (1906). They include grimacing, vocalization, increased heart rate and blood pressure, increased respiratory rate, generalized or regional muscle contractions with non-lateralized visceral stimuli and flexion/withdrawal and head-turning with lateralized somatic stimuli (Woodworth and Sherrington, 1904). These so-called 'pseudoaffective' responses are only triggered by noxious stimuli, cease when the noxious stimulus is removed, and are reflexly mediated by neurons within the brain-stem and spinal cord. They persist in the decerebrated animal preparation (Ness and Gebhart, 1988).

Unlike humans, experimental animals are incapable of reporting and describing their experience to painful stimuli. Consequently, investigators must rely

on other indices of nociception such as motor reflexes, autonomic responses and pain-like behaviours (i.e. scratching, biting, vocalization, escape efforts, hyper-responsiveness to touch). The characteristic patterns of pseudoaffective responses to noxious stimulation have made them a useful and commonly employed measure of nociception in experimental animals (Gebhart and Ness, 1990, Ness and Gebhart, 1990)

The use of pseudoaffective responses to actually characterize a stimulus as "noxious", as suggested by Cervero (1981) , is less reliable. In the cat, blood pressure and single unit electrical activity in viscerosomatic neurons of the thoracic spinal cord were increased by noxious distension of the biliary system, but were unchanged at innocuous distension pressures (Cervero, 1981, 1982a, 1982b). However, pseudoaffective responses can be evoked by visceral stimuli that elicit no behavioral evidence of pain (Ness *et al.*, 1990). Intra-coronary injections of bradykinin (10-300 ng/kg) produced a graded pressor response in unanesthetized dogs without any signs of a painful reaction such as vocalization and agitation (Pagani *et al.*, 1985). The dogs remained calm or somnolent and showed an increase in depth of respiration (Pagani *et al.*, 1985). Thus, pseudoaffective responses are used as an quantitative index of nociception to a stimulus that is known to be noxious. By themselves, pseudoaffective responses are not sufficient to characterize a stimulus as being 'noxious'.

Animal Models of Visceral Pain

It has long been known that visceral organs can be exposed to cutaneous noxious stimuli (i.e. cutting or burning) without evoking pain. Visceral pain occurs when the capsule surrounding an organ is distended or the parenchyma becomes inflamed by pathological processes (i.e. obstruction of the common bile duct triggering severe visceral pain). Thus, an animal model of visceral nociception requires a stimulus that stretches, twists or contracts visceral structures. Ideally, the experimental stimulus should be: a) similar to a natural stimulus; b) minimally invasive; c) controllable; d) reproducible; and e) quantifiable (Ness and Gebhart, 1990). To date, four types of stimuli have been used in basic studies of visceral nociception; electrical, mechanical, ischemic and chemical stimulation.

Electrical stimulation of a nerve or group of nerves innervating a given organ can be reliable and highly reproducible. Unfortunately, it is not specific for any sensory modality. In contrast, interruption of the blood supply to a visceral organ is not a reliable stimulus since the ischemic myocardium or the infarcted bowel is often clinically "silent" and only recognized as abnormal by an alteration in the electrocardiogram (ECG) or the onset of associated symptoms such as peritonitis. The marked variability in the neuronal responses to ischemia, both within and between preparations, also make this type of nociceptive stimulus unreliable (as reviewed by Ness and Gebhart, 1990).

Allogenic chemicals, administered topically to the exposed surfaces of selected organs or injected into their blood supply, have also been used for visceral nociception. However, topical application limits the site of action to a localized area of the organ, and the intra-arterial or i.v. administration may produce widespread activation of the neural afferents in all layers of an organ, depending on the site of injection. In the rodent writhing test, allogenic substances are injected i.p. and the number of evoked 'stretching' or 'writhing' responses, comprised of alternating flexion and extension of the abdominal muscles, is then counted per unit time. Although this test is commonly used for screening antinociceptive drugs, the onset, intensity, and duration of writhing are not easily controlled, the actual source of the "pain" is unknown, and non-analgesic, as well as analgesic, drugs are "efficacious" in this model (Ness and Gebhart, 1990, Gebhart and Ness, 1990). In addition, the stimulus is inescapable and long lasting raising ethical concerns about this model.

Mechanical stimulation of the viscera, such as distension of a hollow organ, stretching of serosal tissue, compression of an organ (i.e. testes), and traction of the mesentery, all produce pain in humans. Pollard and Bloomfield (1931b) studied pain arising from the distension of different regions in the gut of humans. Gastric, duodenal or colonic distension each triggered reports of pain that ranged from 'severe sharp pain' to a 'burning and gnawing pain'. Bentley and Smithwick (1940) also studied pain evoked by distending the jejunum of 6 healthy individuals. At low distension volumes, this pain was roughly localized to the epigastric region but

spread to include the entire abdomen when greater distension volumes were used. These results were confirmed by Ray and Neil (1947). Pain was most intense when long continuous segments of gut were distended simultaneously (Lewis, 1942). Thus, graded distension of a hollow visceral organ (i.e. the gastrointestinal tract, urinary tract, vagina, uterus, biliary tree and gallbladder) mimics a natural and painful stimulus in humans. Unlike the natural stimulus however, the distending pressure is controllable, reproducible and can be easily quantified.

Based on these clinical observations, investigators have used mechanical distension to study visceral nociception in animals. Such studies frequently involve the gut because it can be easily accessed through the mouth or the anus. Noxious mechanical distension of the gut triggers reproducible pseudoaffective cardiovascular (Lembeck and Skofitsch, 1982), respiratory (Satpathy and Al-Sattar, 1984) and visceromotor responses (Ness and Gebhart, 1988) in experimental animals. Distension of middle portion of esophagus has been reported to trigger a depressor response followed by an increase in blood pressure in nembutal-anesthetized dogs (Satpathy and Al-Sattar, 1984). An interesting study by Gayheart *et al.* (1991) showed that esophageal distension in α -chloralose-anesthetized dogs causes coronary vasoconstriction; an effect mediated by α -adrenoceptors. Euchner-Wamser *et al.* (1993) also showed that distension of middle and lower esophagus triggers bradycardia in pentobarbital-anaesthetized rats and found that neurons in T2-T4 spinal dorsal horn, responsive to graded esophageal distension,

exhibited a linear and accelerating stimulus-response function. The type and dose of anaesthetics may affect the direction of cardiovascular responses to the distension of gut (Ness and Gebhart, 1988).

Overall, these data indicate that distension of a visceral organ is an effective noxious stimulus evoking both pain and reflex pseudoaffective responses. In the present study, graded esophageal distension was used as the experimental stimulus, and the evoked pseudoaffective cardiovascular responses were measured in order to investigate the afferent pathway(s) through which this esophago-cardiovascular reflex is triggered in the rat.

Pharmacological Properties of Pseudoaffective Responses to Visceral Stimuli

Pseudoaffective responses to noxious stimulation are physiological reflexes having an afferent and an efferent pathway. Sensory spinal and/or cranial nerves comprise the afferent arm of the reflex. The efferent arm is composed of sympathetic fibers that mediate the autonomic responses to noxious stimulation. Internuncial fibers in the medulla and spinal cord play a vital role in the activation and coordination of these reflexes but their identity and pharmacology are poorly understood. Efforts to characterize these pathways with neuroablative and pharmacological techniques have been attempted using different models of visceral nociception.

In early studies, pseudoaffective visceromotor and cardiovascular reflexes evoked by intestinal manipulation, including pinching the small or large intestine, pinching the pancreas and pulling the mesentery (Miller *et al.*, 1924; 1925 a,b; Lewis and Kellgren, 1939; Downman and McSwiney, 1946) were retained in animals transected below the medulla (Downman and McSwiney, 1946). In a more comprehensive study, the heart rate and pressor responses evoked by graded colorectal distension were not significantly affected by partial sympathectomy (L6-S3 segments) or by mid-collicular decerebration (Ness and Gebhart, 1988) as compared to intact control rats. However, both responses were markedly attenuated or absent in spinalized rats, either acutely at the C1 spinal segment, or chronically at mid-thoracic (T6) spinal level. Similar results were obtained using the visceromotor response to colorectal distension (a contraction of the abdominal and hind limb musculature recorded on the EMG and observed as 'hunching' of the rat). These data indicate that both the cardiovascular and visceromotor responses evoked by colorectal distension are activated by brainstem loops. The loop underlying the pressor and heart rate responses probably involves sympathoexcitatory neurons located in the brainstem. Because surgical demedullation of the adrenal glands significantly reduced the heart rate, but not the pressor response, as compared to sham operated controls, the heart rate response appears to also involve the adrenal glands.

In the same model, Ness and Gebhart (1989) showed that i.v. chlorisondamine (a ganglionic blocker) and phentolamine (a non-selective α -adrenoceptor antagonist), each inhibited the distension-evoked rise in heart rate, and converted the normal pressor response to a slight depressor response. Propranolol or atropine also markedly inhibited the heart rate response, but had little effect on the pressor response. These data indicate that the cardiovascular responses evoked by noxious colorectal distension arise from the reflex activation of sympathetic outflow and the removal of vagal tone. Comparable results were reported by Pittman *et al.* (1988) using gastric distension in anesthetized rats. Indeed, a reflex increase in cardiac sympathetic activity and a concurrent decrease in vagus nerve activity were recorded during urinary bladder distension in anesthetized cats (Taylor, 1968) and dogs (Hassan *et al.*, 1987a,b). Moreover, urinary bladder distension in anesthetized dogs induced a pressure-dependent attenuation of vagus nerve activity in response to incremental increases in carotid sinus pressure (Ramadan *et al.*, 1989). These physiological and pharmacological data are consistent with a reflex modulation of the autonomic nervous system by noxious visceral stimulation.

As a presumptive response to noxious stimulation, pseudoaffective reflexes should be sensitive to antinociceptive drugs. In general, all classes of antinociceptive agents, including α_2 -adrenoceptor agonists, serotonergic agonists and capsaicin, significantly attenuate the pseudoaffective responses to noxious

visceral stimuli. Thus, intrathecal (i.t) and i.v. morphine dose-dependently inhibited the cardiovascular and visceromotor responses to colorectal distension; an effect reversed by naloxone (Ness and Gebhart, 1988). Rat dorsal horn neurons in the L3-L5 segments, activated by noxious colorectal distension, exhibited a comparable sensitivity to morphine and naloxone (Ness and Gebhart, 1989). Antinociceptive synergy between intrathecal morphine and lidocaine has also been shown in the colorectal distension model (Maves and Gebhart., 1992).

However, sensitivity to opioid analgesic drugs is not restricted to the colorectal model. The cardiovascular responses evoked by distension of the proximal jejunum in urethane-anesthetized rats were inhibited by morphine and reversed by naloxone (Clark *et al.*, 1988; Clark and Smith, 1985; Lembeck and Skofitsch, 1982). Interestingly, the threshold intraluminal pressure required to evoke a reflex response was increased by codeine, but not by the quaternary agonist, N-methylmorphine. The effect of codeine was significantly decreased after bilateral vagotomy (Clark and Smith, 1985). Collectively, these results suggest that the opioid effect on the threshold for the reflex is centrally mediated and requires intact vagal innervation. In contrast, the inhibitory effect of opioids on the cardiovascular responses was largely independent of vagal innervation and appeared to involve a peripheral mechanism (Clark and Smith, 1985).

There is abundant evidence that bulbospinal noradrenergic and serotonergic neurons modulate the processing of nociceptive information in the spinal cord (e.g.

Zemlan et al, 1988, Yaksh and Wilson, 1979, Yaksh, 1985, Proudfit 1988).. Consistent with these results, and the role of spinal α_2 -adrenoceptors in noradrenergic antinociception, the α_2 -agonists clonidine and ST-91 inhibited the cardiovascular and visceromotor responses to colorectal distension in dose-dependent, yohimbine reversible fashion following i.t. injection (Danzebrink and Gebhart, 1990;1991b). These inhibitory effects were similar to their actions in animal models of cutaneous nociception (hot plate, tail flick, paw pressure withdrawal test). Single unit recordings of rat dorsal horn neurons, activated by colorectal distension, were also inhibited by intra-arterial clonidine in a dose-dependent, yohimbine-reversible manner (Ness and Gebhart, 1989). Unlike clonidine and ST-91, i.t. tizanidine (another α_2 -adrenoceptor agonist) failed to elevate the visceromotor threshold, despite having a significant inhibitory effect on the pressor response to colorectal distension (Danzebrink and Gebhart, 1990). Tizanidine also increased *resting* mean arterial pressure, suggestive of an effect on cardioregulatory neurons in the spinal cord, or a direct effect on peripheral vascular smooth muscle following systemic redistribution from the spinal subarachnoid space. The ability of α_2 -agonists to inhibit both afferent nociceptive input and efferent sympathetic outflow at the spinal cord level make it important to distinguish between these two effects when assessing antinociceptive activity on the basis of pseudoaffective cardiovascular responses.

In the same model, Danzebrink and Gebhart (1991a) showed that i.t. 5-HT_{1A}, 5-HT_{1B}, 5-HT₂ and 5-HT₃ agonists inhibited the pressor response and elevated the threshold pressure required to evoke the visceromotor response in a dose-dependent manner. The antinociceptive effects of 5-HT, RU-24969 [5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole; a 5-HT_{1B}/5-HT_{1A} agonist], alpha-methyl-5-HT and DOI (5-HT₂ agonists) were antagonized by pretreatment with intrathecal methysergide (a 5-HT₂/5-HT_{1C} antagonist). Pretreatment with intrathecal ketanserin (5-HT₂/5-HT_{1C} antagonist) blocked the effects of MK-212 [6-chloro-2-(1-piperazinyl)-pyrazine; a 5-HT₂ agonist]; MDL-72222 (a 5-HT₃ antagonist) blocked the effects of 2-methyl-5-HT (a 5-HT₃ agonist). Whereas spinal 5-HT₂ receptors appear to mediate serotonergic antinociception in a cutaneous pain model (Solomon and Gebhart, 1988), spinal 5-HT₁, 5-HT₂ and 5-HT₃ receptors inhibit the visceromotor and pressor responses evoked by colorectal distension in the rat.

Finally, primary afferent C-fibers have long been implicated in the transmission of pain (Ganong, 1993). Their selective destruction by the neurotoxin, capsaicin, has made capsaicin a useful pharmacological tool for determining the fiber types mediating noxious sensory transmission (Fitzgerald, 1983, Holzer, 1988) as well as an antinociceptive and clinical analgesic drug (Lynn, 1990). Neonatal treatment with capsaicin reduced the behavioral responses to chemically induced visceral pain and inhibited somato-visceral reflexes in adult rats (Cervero and McRitchie, 1982). Using a similar treatment protocol, capsaicin inhibited the

cardiovascular response evoked by distension of the proximal jejunum in pentobarbital-anesthetized rats (Lembeck and Skofitsch, 1982). Neonatal capsaicin has also been reported to selectively affect the visceromotor response to colorectal distension (Gebhart and Ness, 1990). Thus, in capsaicin-treated rats, the threshold colorectal pressure evoking the visceromotor response was doubled, whereas the cardiovascular responses remained unchanged. Non-steroidal anti-inflammatory agents, such as indomethacin and dipyrene, that are effective in rodent writhing tests, had no significant effect on the cardiovascular responses evoked by noxious distension of the jejunum in urethane-anesthetized rats (Clark *et al.*, 1988).

While not a comprehensive review, data from these representative studies confirm the sensitivity of pseudoaffective responses, triggered by noxious visceral stimuli, to known antinociceptive agents at doses that inhibit cutaneous nociception. They also implicate the role of C-fibers in visceral pain that triggers these reflexes. Overall, these results support the hypothesis that: a) pseudoaffective responses represent nociception-evoked reflexes; and b) that these responses can be used as indices of visceral nociception in experimental animals.

Rationale and Specific Research Objectives

Abnormalities in the esophagus are known to cause chest pain in humans that is difficult to distinguish from the pain of myocardial ischemia. At the present time, the diagnosis of chest pain often requires a complete cardiac workup before

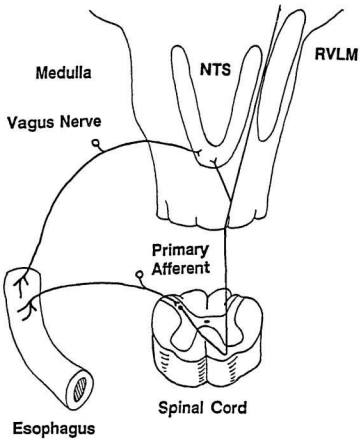


Figure 3. The spinal and vagal afferent innervation of the esophagus. NTS = Nucleus of the solitary tract; RVLM = rostromedial ventral lateral medulla

an esophageal etiology is identified. Underlying this clinical problem is a lack of information about the sensory pathways that convey pain from the esophagus to the CNS (Fig. 3). Thus, spinal and/or vagal afferent fibers from the esophageal may be important in esophageal chest pain.

Graded esophageal distension, using a balloon catheter inserted into the lumen of the esophagus, not only evokes pain in humans, but has been used to evaluate patients with suspected esophageal chest pain (Barish *et al.*, 1986, Paterson *et al.*, 1991). The results of these studies showed that patients with esophageal chest pain are not only sensitive to distension, but have a lower threshold for esophageal pain as compared to control subjects.

Esophageal distension in the rat (Meller and Gebhart, 1991) has been shown to evoke a pattern of reflex responses, subsumed under the term of "pseudoaffective" reflexes (Sherrington, 1906), that are suggestive of a painful event. This may be a useful model for investigating the mechanisms underlying clinical esophageal pain. In the present study, we used urethane anesthetized rats to characterize the physiological and pharmacological properties of esophageal distension-evoked cardiovascular (pressor and cardioaccelerator) responses.

The purposes of this research were to: a) determine whether the relevant sensory input evoking these pseudoaffective responses is conveyed to the central nervous system (CNS) by vagal and/or spinal afferent fibers; and b) study the effects and the sites of action of antinociceptive agents on this reflex. The specific

objectives were:

1. To develop an esophago-cardiovascular reflex model in urethane-anesthetized rats.
2. To determine the most sensitive portion of the esophagus for distension-evoked cardiovascular responses.
3. To determine the relationship between esophageal distension pressure and evoked cardiovascular responses.
4. To determine the effect of vagotomy on the esophago-cardiovascular reflex, and to compare its effect on the responses evoked by cutaneous mechanical and chemical nociceptive stimuli.
5. To determine the effect of neonatal capsaicin on the esophago-cardiovascular reflex and to compare its effect on the responses evoked by cutaneous thermal and mechanical nociceptive stimuli.
6. To compare the effect of morphine, given i.t., i.v. and topically to the surface of the solitary complex, on the esophago-cardiovascular reflex.
7. To compare the effect of dexmedetomidine, given i.t., i.v. and topically to the surface of the solitary complex, on the esophago-cardiovascular reflex.
8. To determine the effect of esophageal paralysis, induced with i.v. N_2 - and M-receptor antagonists, on the esophago-cardiovascular reflex.

MATERIALS AND METHODS

All procedures in this study were approved by the Animal Care Committee of Memorial University of Newfoundland in accordance with the Guidelines of the Canadian Council on Animal Care.

Animals

Male Sprague-Dawley rats (350-450 g at the time of the experiment), and female pregnant rats (15-16 days of gestation on arrival) were obtained from Charles River Inc. (St. Constant, Canada). Animals were housed in groups of 2 per cage at a room temperature of 22°C with a 12-hour light-dark cycle (lights on at 0800 h). Rats with intrathecal catheters, and female rats were housed individually. Purina® rodent laboratory chow and tap water were provided *ad libitum*.

Implantation of Intrathecal Catheters

Intrathecal catheters were constructed from polyethylene (PE-10) tubing and implanted under halothane (Halocarbon Laboratories, North Augusta, USA) anaesthesia. The catheter was inserted through a slit in the cisternal membrane terminating at the mid-thoracic level (T4-T5) or the rostral end of the lumbar enlargement (L1-L2) of the spinal cord. A fixed loop near the rostral tip of the catheter was sutured to the overlying muscle and the incision closed. The rostral end of the catheter was externalized on the top of the head and sealed with a

stainless steel plug. Animals with intrathecal catheters were then housed individually and allowed to recover for a minimum of 5 days. Only those animals without overt signs of neurological impairment were used for experimentation. At the end of the experiment, methylene blue was injected through the intrathecal catheter and the position of the catheter tip was visually determined in the spinal cord. Data from individual animals were excluded if the catheter was not in the subarachnoid space with the tip at the appropriate spinal segment.

Capsaicin Treatment

Capsaicin (Sigma Chemical Co., St. Louis, USA) was dissolved in a mixture of absolute ethanol (Aldrich Chemical, Milwaukee, USA), TWEEN 80 (Willer Fine Chemicals, London, Canada) and 0.9% saline (2:2:16 volume) and injected s.c. on the back of the neck under halothane anaesthesia. Capsaicin was administered at a dose of 25 mg/kg on post-natal day 2, and at 50 mg/kg on post-natal days 3, 4, 11, 25, 55 and 85. Rat pups were returned to their mothers after capsaicin or vehicle treatment. At 6 weeks of age, rats were housed in separate cages.

To confirm the neurotoxic effect of capsaicin on C-fibers, behavioral responses to thermal and mechanical nociceptive stimuli were determined at 3-months of age (before the experiment). Tail flick latency was measured with a tail flick analgesy meter (Model MK-330, Muromachi Kikai Co., Tokyo, Japan) (D'Amour and Smith, 1941). A cutoff time of 10 seconds was used to avoid tissue damage.

Paw pressure withdrawal was measured with a Ugo Basile Analgesy-meter (Biological Research Apparatus, Comerio-Vaese, Italy) (Randall and Seltito, 1957). Pressure was applied to the dorsal surface of the non-inflamed hind paw until a complete withdrawal or withdrawal attempt was observed. The maximum pressure applied to the hind paw was 750 grams.

Surgical Preparation and Physiological Recording

Under halothane anaesthesia, cannulae were surgically placed in the femoral artery, the external jugular vein and trachea for recording arterial blood pressure and heart rate (HR), i.v. drug injection, and mechanical ventilation (vagotomized rats), respectively. The incision was sutured and coated with 2% lidocaine gel (Astra Pharma, Mississauga, Canada). After surgery, halothane was discontinued and anaesthesia was maintained with i.v. urethane (0.8 g/kg + 20 mg/kg/h). Urethane (Sigma Chemical Co.) was dissolved in sterile saline and administered as a 10% w/v solution. Body temperature was maintained at 37-38°C throughout the experiment using a thermostatically controlled heating blanket (Harvard Instruments, St. Laurent, Canada). Arterial blood pressure and HR were continuously monitored using a Statham pressure transducer coupled to a Gould 8000S Recorder which was equipped with an ECG/Biotach amplifier. The EKG was recorded on a polygraph (model 7P1, Grass Instruments, Quincy, USA) with input leads on the right and left arms and the isolated ground electrode on the right leg. Respiration

was monitored on the polygraph using a temperature sensitive transducer (model TCR-1R, Grass Instruments) inserted in the upper third of the tracheal catheter.

A small balloon made from PE-50 tubing was filled with water and placed in the lower esophagus (10.5-11.0 cm from the incisors). In some experiments, similar balloons were inserted in the upper and middle esophagus (5.0-5.5 cm and 8.0 cm from incisors, respectively). Animals were allowed to stabilize for 30 minutes before experimentation. The esophagus was distended by inflating the balloon with water using a hand held syringe. The resulting esophageal distension pressure was calibrated with a manometer or measured using a pressure transducer coupled to the polygraph.

In those experiments where cervical vagotomy was performed, rats were mechanically ventilated through a tracheal catheter with a small animal respirator (Harvard Instruments). The ventilation rate was 100 strokes per minute with a tidal volume of 2 ml per 100 grams of body weight (maximum volume of 8 ml). The cervical vagi were separated from the carotid arteries and sympathetic nerves, and severed at the level of the larynx.

Noxious Mechanical and Chemical Stimulation

Two types of noxious stimuli were used; calibrated paw pinch (Sherman and Loomis, 1994) and the topical application of xylene (Olsen and Lund, 1991). To apply the paw pinch, the left paw was gripped with a hemostat at the post axial

border (on the medial surface, distal to the tarsal joint), such that the region of skin covered by the hemostat was about 20 mm². The force of the pinch was produced by 500 gram-weights attached to the handles of the hemostat. This resulted in final pressure on each paw of approximately 0.7 kPa. Reflex withdrawal of the hind paw was prevented until the end of the 20 s stimulus period. Noxious chemical stimulation was produced by the application of 10 µl of mixed xylene (Sigma Chemical Co.) on the plantar surface of one front paw (before or after unilateral vagotomy) followed by one hind paw (after bilateral vagotomy).

Drug Administration

All drugs, except capsaicin, were dissolved in 0.9% saline and doses are expressed as their salts. Drugs were injected i.v. in a volume \leq 0.1 ml followed by a 0.1-ml saline flush. Intrathecal drugs were injected through the spinal catheter in a volume of 6 µl and flushed with 10 µl of sterile saline. For medullary drug administration, the rat was placed in a stereotaxic apparatus and the dorsal surface of the solitarius complex and area postrema was exposed. Drugs were applied manually in volumes of 0.02-0.05 µl from a Hamilton microliter syringe (1 µl) under microscopic control.

Immunohistochemistry

At the end of the experiments, substance P (SP)- and calcitonin gene related

peptide (CGRP)-like immunoreactivity (IR) were determined in the esophagi of vehicle- and capsaicin-treated animals to confirm the neurotoxic effect of capsaicin. Rats were injected with an overdose of i.p. urethane and the esophagus was rapidly removed. The esophagus was flushed with phosphate buffered saline (PBS), tied with a ligature at the rostral end, and filled with 0.4% paraformaldehyde (0.8-1.0 ml). The caudal end of the esophagus was closed with another ligature and fixation allowed to continue for 30-45 minutes. The esophagus was cut longitudinally and the striated and smooth muscle layers were separated.

The esophagus was then: 1) washed with PBS for 20 minutes; 2) immersed in 50% alcohol for 5 minutes, 70% alcohol for 5 minutes, 100% alcohol for 10 minutes and xylene for 30-40 seconds; 3) immersed in 100% alcohol for 10 minutes, 70% alcohol for 10 minutes, 50% alcohol for 10 minutes, and PBS; 4) washed four times with PBS (30-40 ml for 30 min per wash) using a mechanical shaker (modified from Costa and Furness, 1983).

Whole esophageal mounts were processed for SP- or CGRP-like IR employing the peroxidase-antiperoxidase (PAP) method (Sternberger, 1979). Tissues were incubated in 10% normal goat serum (ICN Biomedicals, Toronto, Canada) containing 0.3% H_2O_2 and 0.4% Triton X-100 for 1 h, followed by rabbit antiserum to SP (1:2000 dilution, Incstar Corp., Stillwater, USA) or CGRP (1:10,000 dilution; Amersham Canada Ltd., Oakville, Canada) for 24 h at room temperature and at 4°C for a further 24 h. They were then: washed four times with PBS (as

described above); dried; incubated with 1:150 dilution of goat-anti-rabbit-IgG serum (Boehringer Mannheim Biochemicals, Indianapolis, USA) for 3 h at room temperature; and stored overnight at 4°C. After washing with PBS, the tissues were incubated with a 1:300 dilution of rabbit PAP (Sternberger Monoclonals Inc., Baltimore, USA) for 3 h at room temperature and at 4°C overnight, washed again with PBS and incubated in a staining medium containing 3',3' diamino-benzidine HCl (0.5 mg/ml, Sigma Chemical Co.), glucose oxidase (3.8 U/ml, Aspergillus niger type V, Sigma Chemical Co.) and D-glucose (2 mg/ml, Sigma Chemical Co.) in 0.1 M phosphate buffer (pH 7.2).

Other Drugs

Methylene blue, scopolamine bromide, and methscopolamine bromide (Sigma Chemical Co., St. Louis, USA), morphine sulphate (BDH Chemicals, Toronto, Canada), and naloxone hydrochloride (Research Biochemicals Inc., Natick, USA) were purchased from commercial suppliers. Wyeth 27127 (Wyeth Ltd., Philadelphia, USA), dexmedetomidine hydrochloride (Orion Corporation Farmos, Turku, Finland), and pancuronium bromide (Organon Canada Ltd., Toronto, Canada) were generously supplied by their manufacturers.

Data Analysis

All cardiovascular data are presented as the maximum **change** in mean

arterial pressure (Δ MAP) and heart rate (Δ HR) during esophageal distension relative to the immediate pre-stimulus (control) period. Mean arterial blood pressure (MAP) was calculated from the following equation:

$$\text{MAP} = \text{Diastolic blood pressure} + 1/3 \text{ pulse pressure}$$

where pulse pressure is the difference between systolic and diastolic pressure. Variability associated with single measurements is expressed as the standard error of the mean (SEM). Variability associated with blocks of data is indicated by pooled 95% confidence intervals. The ED_{50} and 95% confidence interval of morphine or DX was calculated from their respective dose-response curve using the method of Tallarida and Murray (1987). Significant differences among multiple treatment groups were determined using one-way analysis of variance (ANOVA) followed by the Newman-Keuls test. A Student's t-test was used to compare differences between two groups. Two-tailed, unpaired Mann-Whitney or Wilcoxon tests were used when there was heterogeneity of variance between two groups. The relationship between esophageal distension pressure and cardiovascular responses was determined by linear regression analysis.

RESULTS

The relationship between esophageal distension pressure and cardiovascular responses

Graded esophageal distension (20 second duration) in urethane-anesthetized rats evoked a pressor and tachycardic response, hereafter referred to as the esophago-cardiovascular reflex, that was time-linked to the stimulus (Fig. 4). MAP and HR increased linearly with the log of the distension pressure over the range 25-150 mmHg (Fig. 5). As indicated by the SEM and 95% CI, the monotonic stimulus-response relationships for MAP and HR were highly reproducible (Fig. 5). The preparation was also sensitive to esophageal distension as indicated by the relatively low threshold pressures yielding detectable and quantitative cardiovascular changes (Table I).

Table I. Esophageal Distension Threshold (mmHg) for Δ MAP and Δ HR

	Distension Threshold*	95% Confidence Interval
Δ MAP	10.2	8.63-12.2
Δ HR	11.4	8.73-14.9

* Estimated from the mean stimulus-response curves (n=7) using the linear transformation:

$$\Delta\text{MAP} = (\log \text{distension pressure})(\text{slope}) + \log \text{threshold pressure}$$

Respiration was reduced during esophageal distension (Fig. 6). Respiratory frequency, averaged over 20 sec before and during esophageal distension in each

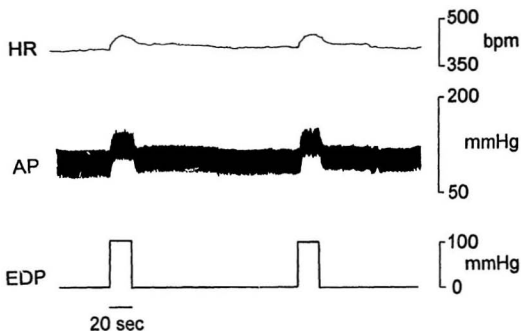


Figure 4. Representative heart rate (HR) and mean arterial blood pressure (AP) responses to lower esophageal distension in urethane-anesthetized rats. Inflation was applied to the distal esophagus, 10.5-11.0 cm from incisors, at a pressure of 100 mmHg for 20 seconds. EDP = esophageal distension pressure

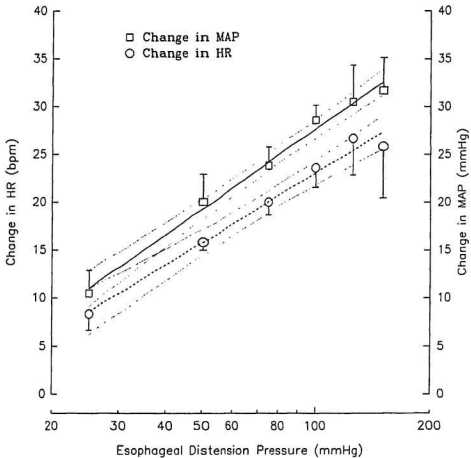


Figure 5. The relationship between esophageal distension pressure and evoked cardiovascular responses. The maximum change in mean arterial pressure (MAP; \square) and heart rate (HR; \circ) following graded esophageal distension is shown. The distension balloon was located in the lower esophagus, 10.5-11.0 cm from the incisors. Each point represents the mean \pm SEM of 6-7 urethane-anesthetized rats. The least squares regression line and corresponding 95% confidence intervals (dotted lines) are shown.

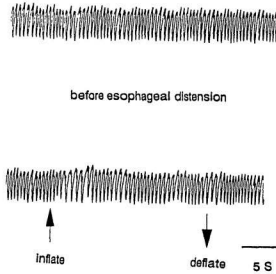


Figure 6. Distension of the lower esophagus reduces the frequency of respiration. Esophageal distension was applied at a pressure of 100 mmHg for 20 sec. Respiration was most affected during the first 5 sec of distension.

of 6 rats, was significantly lower during esophageal distension ($p < 0.0001$; data not shown). Except for the increase in heart rate, esophageal distension did not cause any EKG wave change or atrioventricular or sinus node block (Fig. 7).

The effect of balloon position on the esophago-cardiovascular reflex

The position of the balloon along the length of the esophagus affected the magnitude of the cardiovascular responses evoked by esophageal distension. Using a fixed pressure stimulus (100 mmHg for 20 s), the cardiovascular responses to lower (10.5–11.0 cm from incisors) and middle (8.0 cm from incisors) esophageal distension were significantly greater than those evoked by distension of the upper esophagus (5.5–6.0 cm from incisors) (Fig. 8). Therefore, lower esophageal distension was used in the remainder of the experiments. While not significant, there was a trend towards increased reflex responses in the lower as compared to the middle esophagus.

The pressor and heart rate responses evoked by esophageal distension depend on the integrity of vagal innervation

As shown in Fig. 9, unilateral vagotomy attenuated the cardiovascular responses to esophageal distension by about 50% of control. Bilateral cervical vagotomy completely abolished this reflex. In contrast, vagotomy had no significant effect on the change in MAP evoked by cutaneous mechanical or chemical noxious



before esophageal distension



during esophageal distension

Figure 7. Esophageal distension had no detectable effect on the EKG except for an increase in heart rate.

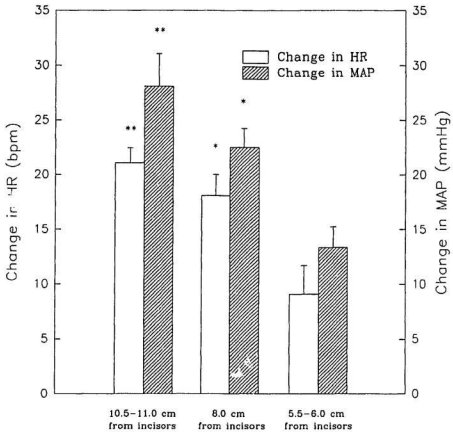


Figure 8. The position of the balloon in the esophagus affects the distension-evoked change in mean arterial pressure (Δ MAP) and heart rate (Δ HR). The balloons were inserted in the esophagus 5.5-6.0 cm, 8.0 cm, and 10.5-11.0 cm from the incisors. Esophageal distension was applied at a pressure of 100 mmHg for 20 seconds. Each point represents the mean \pm SEM of 6-8 rats. The change in MAP and HR evoked by lower and middle esophageal distension is significantly greater than that evoked by distension in the upper esophagus (* $P < 0.05$; ** $P < 0.01$).

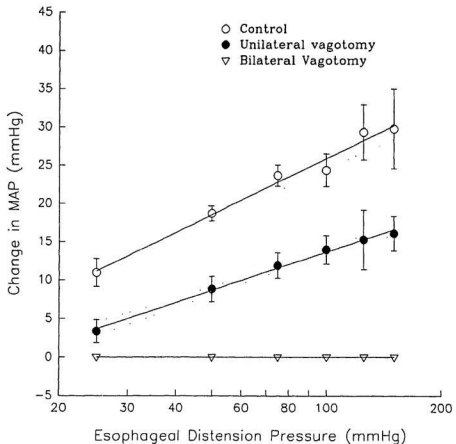


Figure 9A. The change in mean arterial pressure (Δ MAP) evoked by esophageal distension depends on the integrity of vagal innervation. The maximum change in mean arterial pressure (MAP) following esophageal distension was recorded in each animal before (open circles), after unilateral (solid circles) and after bilateral vagotomy (triangles). Each point represents the mean \pm SEM of 7 rats. Least squares regression line and 95% confidence intervals (dotted lines) are shown. Inflation was applied to the distal esophagus, 10.5 to 11 cm from the incisors.

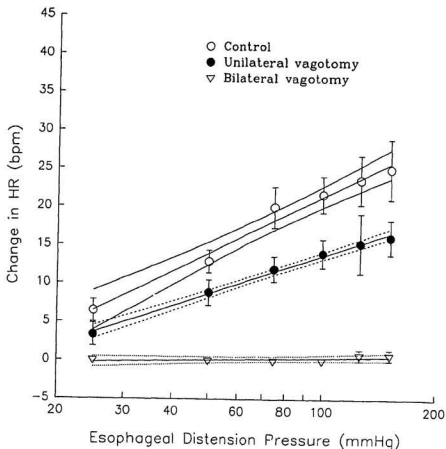


Figure 9B. The change in heart rate (Δ HR) evoked by esophageal distension depends on the integrity of vagal innervation. The maximum change in heart rate (HR) following esophageal distension was recorded in each animal before (open circles), after unilateral (solid circles) and after bilateral vagotomy (triangles). Each point represents the mean \pm SEM of 7 rats. Least squares regression line and 95% confidence intervals (dotted lines) are shown. Inflation was applied to the distal esophagus, 10.5 to 11 cm from the incisors.

stimulation (Table II). There was also no significant difference in the HR response to noxious stimulation between normal and unilaterally vagotomized animals. Because the basal HR increased after bilateral vagotomy, the change in HR evoked by paw pinch or topical xylene was significantly reduced as compared to control or unilateral vagotomy.

Table II. The Effect of Vagotomy on the Cardiovascular Responses to Noxious Mechanical and Chemical Stimulation

Treatment	N	Δ MAP (mmHg)	Δ HR (bpm)
Hind Paw Pinch			
Control	5	18.5 ± 1.2	23.0 ± 6.4
Unilateral Vagotomy	5	17.0 ± 2.3	34.5 ± 4.1
Bilateral Vagotomy	5	12.0 ± 2.3	$12.0 \pm 3.0^*$
Topical Xylene			
Control	5	21.3 ± 5.4	26.0 ± 6.0
Unilateral Vagotomy	5	21.3 ± 4.9	34.0 ± 4.8
Bilateral Vagotomy	5	16.3 ± 3.3	$11.0 \pm 4.0^*$

* $P < 0.05$ as compared to unilateral vagotomy.

The esophago-cardiovascular reflex persists after esophageal paralysis

Because both sensory afferent and motor efferent fibers of the esophagus were transected in the experiment described above, the failure to observe the esophago-cardiovascular reflex may be due to esophageal paralysis, or at least a change in the stimulus-response relationship secondary to esophageal relaxation.

To test this possibility, esophageal paralysis was induced pharmacologically in a separate group of rats with the muscarinic (M)-receptor antagonists, methscopolamine (0.2 $\mu\text{mol/kg}$, i.v.) and scopolamine (0.2 $\mu\text{mol/kg}$, i.v.) alone, or in combination with the N_2 -receptor antagonist, pancuronium (200 $\mu\text{g/kg}$, i.v.). None of these treatments significantly affected the pressor response to esophageal distension (Table III). However, pancuronium seems to have a inhibitory effect on pressor response to esophageal distension though it is not statistically significant. Anticholinergic-induced tachycardia occluded the HR response to esophageal distension.

Table III. The Pressor Reflex Persists After Esophageal Paralysis

Treatment	N	Δ MAP (mmHg)	Δ HR (bpm)
Control	6	23.1 \pm 3.2	15.7 \pm 1.4
Methscopolamine (0.2 $\mu\text{mol/kg}$ i.v.)	6	24.1 \pm 3.4	6.9 \pm 2.2*
" + Scopolamine (0.2 $\mu\text{mol/kg}$ i.v.)	6	20.4 \pm 2.7	4.7 \pm 1.8**
" + Pancuronium (200 $\mu\text{g/kg}$ i.v.)	6	15.4 \pm 2.6	6.8 \pm 2.2**

Esophageal distension was applied at a pressure of 100 mmHg for 20 seconds. *

P<0.05 ** P<0.01

Neonatal capsaicin partially attenuates the esophago-cardiovascular reflex

The cardiovascular responses evoked by esophageal distension (25-150

mmHg for 20 s) were significantly but incompletely reduced in adult rats that had been treated neonatally with capsaicin as compared to vehicle ($P<0.05$)(Fig. 10). There was no difference between vehicle-treated rats and age-matched (untreated) controls. All capsaicin-treated rats had significantly elevated tail flick latencies ($P<0.0001$; Fig. 11), and paw pressure withdrawal thresholds ($P<0.05$; Fig. 12) as compared to vehicle controls, consistent with the neurotoxic effect of capsaicin on small diameter C-fibers. This effect was confirmed at the end of the experiment by the marked depletion of CGRP- and SP-immunoreactivity from the nerve terminals of small diameter fibers in the esophagi of capsaicin-treated rats (data not shown). These results indicate that the relevant sensory information triggering the esophago-cardiovascular reflex under study is conveyed, in part, by small diameter, nociceptive C-fibers.

Supraspinal, but not spinal, morphine inhibits the esophago-cardiovascular reflex

The effect of morphine, delivered i.v., i.t., or topically to the surface of the solitary complex, is summarized in Table IV. Intravenous morphine significantly attenuated the cardiovascular responses to esophageal distension in a dose-dependent, naloxone-reversible fashion. The ED_{50} and 95% CI values for i.v. morphine were 3.46 mg/kg (2.26-5.32) for the pressor response and 1.99 mg/kg (1.31-3.03) for the heart rate response. A similar effect on MAP was observed when

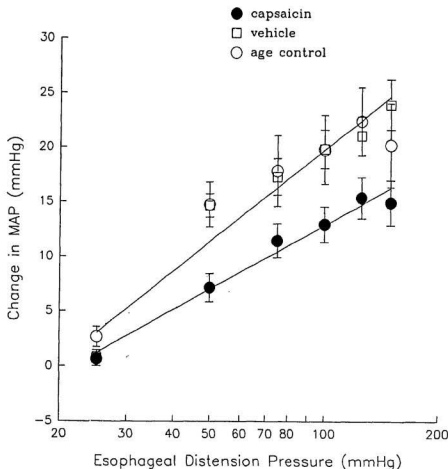


Figure 10A. Neonatal capsaicin partially attenuates the blood pressure response to esophageal distension. The maximum change in mean arterial pressure (MAP) after esophageal distension was recorded in neonatal capsaicin-treated (solid circles), vehicle-treated (squares), and untreated, age-matched control (open circles) rats. The data are expressed as mean \pm SEM of 6-8 rats. The least squares regression line and 95% confidence intervals are shown. For clarity, the confidence intervals for the age-matched control group have been omitted.

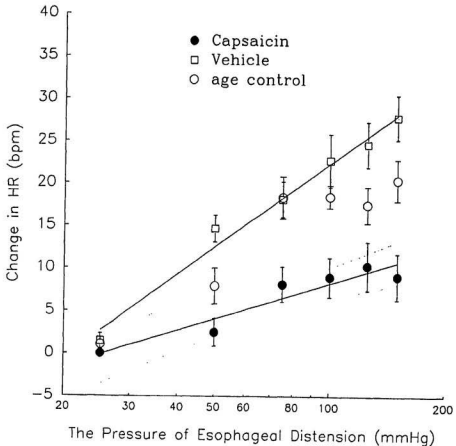


Figure 10B. Neonatal capsaicin partially attenuates the heart rate response to esophageal distension. The maximum change in heart rate (HR) after esophageal distension was recorded in neonatal capsaicin-treated (solid circles), vehicle-treated (squares), and untreated, age-matched control (open circles) rats. The data are expressed as mean \pm SEM of 6-8 rats. The least squares regression line and 95% confidence intervals are shown. For clarity, the confidence intervals for the age-matched control group have been omitted.

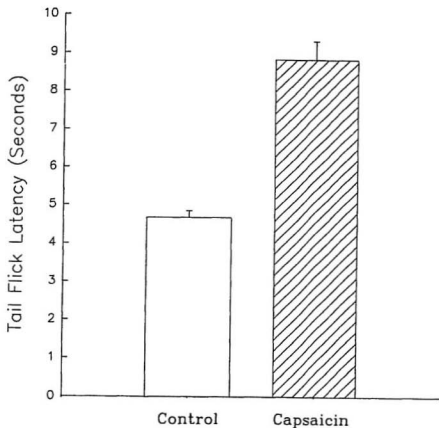


Figure 11. The effect of neonatal capsaicin on peak tail flick latency in adult rats. Data are expressed as mean \pm SEM of 11 rats treated with neonatal capsaicin and 21 rats treated with vehicle. All rats were 3 months of age at the time of the experiment. Neonatal capsaicin significantly increased the tail flick latency as compared to control ($P < 0.0001$).

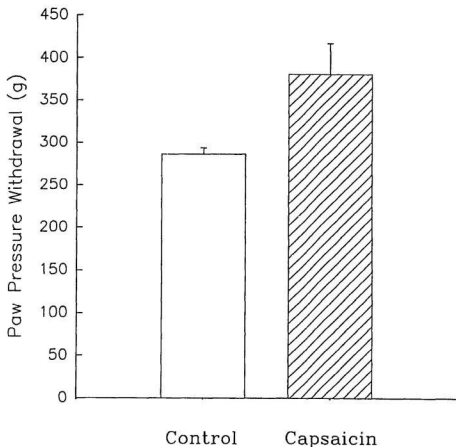


Figure 12. The effect of neonatal capsaicin on peak paw pressure withdrawal threshold in adult rats. Data are expressed as mean \pm SEM of 11 rats treated with neonatal capsaicin and 20 rats treated with vehicle. All rats were 3 months of age at the time of the experiment. Neonatal capsaicin significantly increased the mechanical nociceptive threshold as compared to control ($P < 0.05$).

Table IV. Supraspinal, but not Spinal, Morphine Inhibits the Esophago-Cardiovascular Reflex

Morphine	N	Δ MAP (mmHg)	Δ HR (bpm)
Intravenous			
Control	13	28.3 \pm 2.1	26.3 \pm 2.5
3 μ mol/kg	13	21.0 \pm 1.7*	17.2 \pm 2.4
6 μ mol/kg	13	16.9 \pm 1.5***	13.1 \pm 2.2**
12 μ mol/kg	13	13.5 \pm 1.1***	9.1 \pm 1.7***
+NX 8.2 μ mol/kg	6	24.6 \pm 2.8	21.1 \pm 2.5
Intrathecal (T4-T5)			
Control	6	22.3 \pm 1.7	18.4 \pm 2.1
12 nmol	6	20.3 \pm 2.0	19.6 \pm 3.1
24 nmol	6	18.4 \pm 2.6	17.1 \pm 3.5
48 nmol	6	17.8 \pm 2.9	15.6 \pm 6.8
Topical (Solitarius Complex)			
Control	5	21.1 \pm 1.9	
0.1 nmol	5	11.5 \pm 2.4*	
1.0 nmol	5	3.0 \pm 1.1***	
+ NX 1.0 nmol	5	15.0 \pm 1.9	

Esophageal distension was applied at a pressure of 100 mmHg for 20 seconds. NX = naloxone, * P<0.05 ** P<0.01 ***P<0.001

morphine (0.1 nmol-1.0 nmol) was applied topically to the solitarius complex (Fig. 13). The ED_{50} and 95% CI of topical morphine was 0.13 nmol (0.046-0.362). Baseline mean arterial pressure was not affected by topical morphine. In contrast, morphine (12-48 nmol) injected into the spinal subarachnoid space near the fourth and fifth thoracic segments had no effect. These data indicate that the esophago-cardiovascular reflex is inhibited by antinociceptive doses of morphine acting at supraspinal, but not spinal, μ -receptors.

Supraspinal and spinal dexmedetomidine inhibits the esophago-cardiovascular reflex

To further examine the pharmacology of the esophago-cardiovascular reflex, the α_2 -adrenoceptor agonist, dexmedetomidine (DX) was injected i.v., i.t. and topically to the solitary complex (Table V). Intrathecal DX, injected near the fourth and fifth **thoracic** spinal segments, significantly inhibited the pressor and HR responses to esophageal distension. This effect was dose-dependent, and reversed by the α_2 -selective antagonist, Wyeth 27127 (thoracic i.t.). The ED_{50} and 95% CI was 0.365 μ g (0.081-1.64) for the pressor response and 0.094 μ g (0.04-0.22) for the HR response. Basal HR and MAP were also significantly and dose-dependently reduced by i.t. DX delivered at these spinal segments (Fig. 14 & 15). In contrast, i.t. DX, injected near the first and second lumbar segments, had no effect on the distension-evoked pressor response. Although basal HR and MAP were significantly

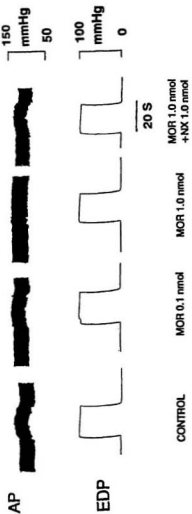


Figure 13. Morphine, applied topically to the solitary complex (NTS), inhibits the esophago-cardiovascular reflex.

reduced by i.t. DX, injected near the lumbar enlargement, and a high dose of DX (2.0 nmol) significantly inhibited the HR response to esophageal distension, these effects were reduced compared to those observed with thoracic i.t. DX.

When low-moderate i.t. doses of DX were given i.v., no effect on the esophago-cardiovascular reflex was observed. Only the highest i.t. dose of DX (2.0 nmol), given i.v., significantly inhibited the distension-evoked HR, but not the pressor response. Baseline HR and MAP were significantly and dose-dependently decreased by i.v. DX (Fig. 14 & 15).

Topical administration of DX (0.1-1.0 nmol) to the solitary complex inhibited the distension-evoked pressor response (Table V), as well as lower esophageal peristalsis (data not shown). Both effects were completely inhibited by 1.0 nmol of DX, and reversed by the topical administration of Wyeth 27127. The ED_{50} and 95% CI was 0.0033 nmol (0.0005-0.022). Topical DX also decreased baseline MAP by 5-15 mmHg (data not shown).

Esophageal inflation induces peristalsis that modulates arterial blood pressure

Distension of the lower esophagus (40 mm Hg) induced rhythmic peristalsis (Fig. 16). Each consecutive wave of esophageal peristalsis was followed 2-4 beats later by a rise and fall in arterial blood pressure. Both esophageal peristalsis and its modulation of the pressor reflex were inhibited in a dose-dependent manner by

Table V. Supraspinal and Spinal Dexmedetomidine Inhibits the Esophago-Cardiovascular Reflex

Dexmedetomidine	N	Δ MAP (mmHg)	Δ HR (bpm)
Intravenous			
Control	8	23.6 \pm 1.8	20.5 \pm 2.4
0.2 nmol	8	23.2 \pm 1.7	18.4 \pm 2.7
0.4 nmol	8	21.5 \pm 2.1	17.2 \pm 2.1
2.0 nmol	8	19.1 \pm 2.1	6.8 \pm 2.0*
Intrathecal (T4-T5)			
Control	7	30.0 \pm 3.9	38.8 \pm 8.5
0.2 nmol	7	22.3 \pm 3.6	21.5 \pm 7.4
0.4 nmol	7	18.9 \pm 3.2*	12.0 \pm 4.3*
2.0 nmol	7	14.1 \pm 2.7*	3.3 \pm 2.1**
+ WY 2.3 nmol	7	25.2 \pm 3.4	18.5 \pm 6.2
Intrathecal (L1-L2)			
Control	8	28.9 \pm 2.6	20.1 \pm 2.3
2.0 nmol	8	26.1 \pm 2.7	12.6 \pm 1.6*
Topical (Solitarius Complex)			
Control	5	17.4 \pm 2.2	
0.1 nmol	5	3.5 \pm 0.6***	
1.0 nmol	5	0***	
+ WY 1.0 nmol	4	11.9 \pm 1.4	

Esophageal distension was applied at a pressure of 100 mmHg for 20 seconds.

WY = Wyeth 27127, * P<0.05 ** P<0.01 ***P<0.001

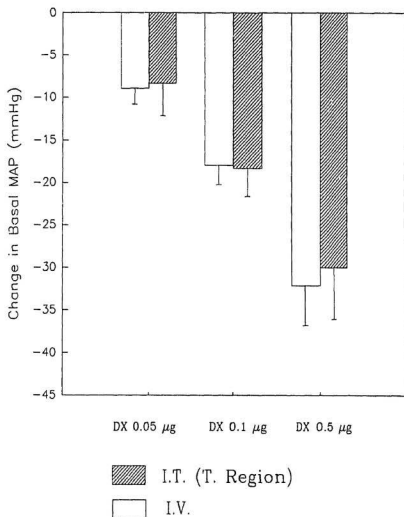


Figure 14. Dexmedetomidine (DX) dose-dependently decreased basal mean arterial pressure (MAP). MAP was continuously recorded before and after i.v. or i.t. DX (no esophageal distension). Intrathecal DX was injected near the mid-thoracic (T region) spinal cord. Data are expressed mean SEM of 7-8 rats (350-450g). For each dose, there was no significant difference between i.v. and i.t. DX.

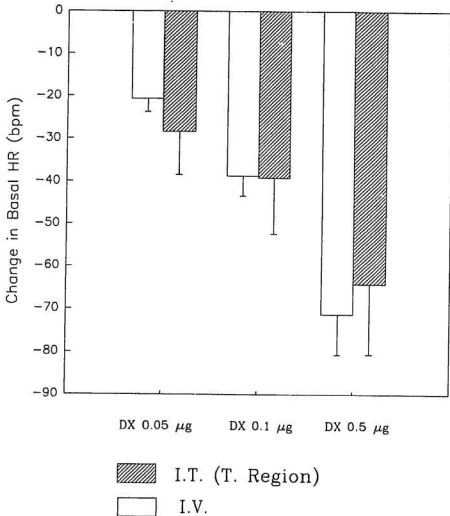


Figure 15. Dexmedetomidine (DX) dose-dependently decreased basal heart rate (HR). HR was continuously recorded before and after i.v. or i.t. DX (no esophageal distension). Intrathecal DX was injected near the mid-thoracic (T region) spinal cord. Data are expressed mean SEM of 7-8 rats (350-450g). For each dose, there was no significant difference between i.v. and i.t. DX.

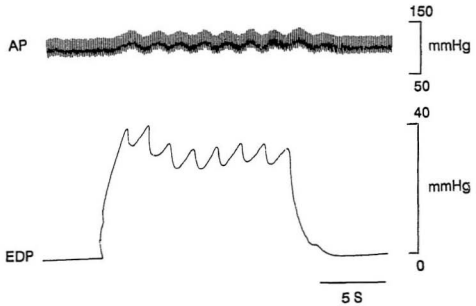


Figure 16. Distension of the lower esophagus evokes esophageal peristalsis that modulates the reflex pressor response. The distal esophageal balloon, located 10.5 to 11 cm from the incisors, was inflated by injecting 20 μ l of water for a period of 15 sec.

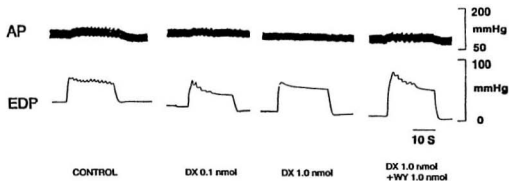


Figure 17. Dexmedetomidine, applied topically to the solitary complex (NTS), inhibits esophageal distension-evoked peristalsis and modulation of the reflex pressor response. Dexmedetomidine and Wyeth 27127 were topically applied to the surface of the NTS. The distal esophageal balloon, located 10.5 to 11 cm from the incisors, was inflated by injecting 20 μ l of water for a period of 15 sec.

the topical application of dexmedetomidine (0.1-1.0 nmol) to the solitary complex (NTS); an effect reversed by Wyeth 27127 applied to the same site (Fig. 17). A similar inhibitory effect was observed with morphine (0.1-1.0 nmol) applied to the NTS (data not shown). This was reversed by the topical application of naloxone to the NTS. However, esophageal peristalsis was not required for the pressor reflex since the latter persisted after esophageal paralysis (Table II).

When the esophageal distension pressure was increased, esophageal peristalsis, and modulation of the pressor reflex (blood pressure waves), were inhibited. The threshold for esophageal peristalsis was about 15 mmHg, while the threshold for unmodulated (non-pulsatile) cardiovascular responses was about 5-10 times higher than that of esophageal peristalsis.

DISCUSSION

In humans, esophageal distension or hypermotility can trigger pain that is difficult to differentiate clinically from cardiac pain. In this regard, the distension of hollow viscera, including the esophagus, is known to evoke a range of autonomic visceromotor and behavioral responses subsumed under the term "pseudoaffective" reflex. In the present study, we have established an esophago-cardiovascular reflex model in the rat and characterized its physiological and pharmacological properties. Specifically, the data obtained provide information about: 1) the reproducibility of the esophago-cardiovascular reflex in the urethane-anaesthetized rats; 2) the pathway by which information triggering this reflex is conveyed to and from the CNS; 3) the dissociation of esophageal peristalsis from the esophago-cardiovascular reflex; and 4) the effects of the antinociceptive drugs, DX and morphine, on this reflex. From these data, we have postulated the possible esophago-cardiovascular reflex arc.

Methodological considerations and relevance of the model for studying esophageal pain.

As the P.E. balloons used in the present experiments were of high compliance, and their diameters (0.6–0.8 cm) exceeded that of the esophageal lumen, the pressure exerted on the esophageal wall closely approximated the intra-balloon pressure. Esophageal distension was easily controlled, reproducible, and the evoked cardiovascular response was graded with increasing esophageal

distension pressure. The intra-luminal balloons were also easily positioned in the esophagus without chest or abdominal surgery. Lower esophageal distension evoked the greatest reflex responses, probably reflecting the increased thickness of the esophageal wall in the distal segment, and/or differences in the type and distribution of visceral nociceptors along the length of the esophagus.

Both the dose and type of general anesthetics have been shown to modify the pseudoaffective cardiovascular and visceromotor reflexes induced by colorectal distension (Ness and Gebhart, 1988). This visceral stimulus was reported to increase arterial blood pressure and heart rate in awake, or alphaxalone/alphadolone acetate (Saffan)-anaesthetized rats but decreased both variables in pentobarbital or α -chloralose-anaesthetized rats (Ness and Gebhart, 1988). In pentobarbital-anaesthetized rats, esophageal distension caused bradycardia and a decrease in arterial blood pressure (Euchner-Wamser *et al.*, 1993). While the heart rate response to colorectal distension (80 mmHg 20 sec) was very small, and hypotension persisted with colorectal distension in urethane-anaesthetized (0.78-0.8 g/kg, i.p.) rats (Ness and Gebhart, 1988), the present observations showed sustained hypertension and tachycardia to esophageal distension in the urethane-anaesthetized (0.8g/kg +20 mg/kg/h) rats.

Esophageal distension in humans is known to cause chest pain. Retrosternal pain radiating to the back, abdominal pain in the upper right quadrant, throat pain, and/or back pain have been reported (De Caestecker *et al.*, 1992). In a study of

patients with noncardiac chest pain, 60% experienced pain at an esophageal balloon inflation volume of 10 ml compared with only 20% of normal subjects (DeVault and Castell, 1992). Not only did these patients develop pain more frequently, but their pain occurred at smaller inflation volumes, suggesting a lower pain threshold to esophageal distension.

Esophageal distension occurs naturally during swallowing, and under normal conditions, this does not cause any change in cardiovascular activity. However, hypotension and loss of consciousness (associated with cardiac slowing, standstill, sinus or atrioventricular block) can be triggered by a swallow in patients with swallowing syncope. This condition, believed to be the result of a pathological vagovagal reflex mediated by tension receptors in the esophageal wall (Ortiz de Murua *et al.*, 1992), can be provoked in susceptible individuals by inflating a balloon in the lower esophagus (Ickrath *et al.*, 1988). Although most patients with swallowing syncope have esophageal disorders, such as malignancies (Tomlinson and Fox, 1975) or spasm (Bortolotti *et al.*, 1982), some have no detectable esophageal pathology (Kadish *et al.*, 1986, Ausubel and Gitler, 1987). The fact that swallowing syncope is so rare and that esophageal disorders are much more frequent, suggests that abnormal central processing is necessary in swallowing syncope. On the other hand, mechanical compression of the heart or large veins may also play a role in this condition. In the present study, distal esophageal distension had no significant effect on the EKG, apart from an increase in heart rate.

Is the esophago-cardiovascular reflex suitable for studying esophageal nociception?

By themselves, the pseudoaffective responses are not sufficient to characterize a stimulus as being 'noxious'. However, they can and are used as a quantitative index of nociception to a visceral stimulus that is known to be noxious. Nociceptive visceral responses generally have pressure thresholds for activation that are higher than those seen for non-pathologic responses (i.e. peristalsis). In our studies, the pressure threshold of the esophago-cardiovascular reflex was approximately 10 mmHg. This is probably well within the physiological range for reflex peristalsis. Distal esophageal distension pressure beyond the physiological range is presumably nociceptive. Whether this represents the actual nociceptive threshold in conscious rats remains to be determined.

Concomitant with the cardiovascular response and esophageal peristalsis, esophageal distension (100 mmHg for 20 sec) also caused a significant decrease in the respiratory frequency, especially during the initial five seconds of esophageal distension. This can be considered as respiratory pseudoaffective reflex response to esophageal distension. As with any other pseudoaffective response, its occurrence *per se* does not define a stimulus as 'noxious'. Esophageal distension occurs naturally and does not evoke a change in respiration unless it is preceded by swallowing. In normal humans, Clark (1920) showed that during swallowing respiration paused for about average 1.4-1.5 seconds, and then resumed, which can

be considered a physiological coordination (Monges *et al.*, 1978).

Is the sensory information triggering the esophago-cardiovascular reflex conveyed to the CNS through vagal or spinal afferents?

The esophagus is supplied by both sympathetic and vagus nerves. Since the cardiovascular response to esophageal distension (25-150 mmHg for 20 s) was attenuated by unilateral vagotomy and abolished by bilateral vagotomy, the afferent limb of the esophago-cardiovascular reflex is likely to be made up of vagal fibers. The finding that thoracic spinal administration of morphine (12-48 nmol), which inhibited the cardiovascular responses to noxious cutaneous mechanical stimulation, failed to inhibit the esophago-cardiovascular reflex supports the argument that all information needed for this reflex was conveyed to the CNS through vagal afferents. Although some spinal neurons (T2-T4) have been shown to respond to esophageal distension in pentobarbital-anaesthetized rats (Euchner-Wamser *et al.*, 1993), spinal afferents do not appear to contribute to the esophago-cardiovascular reflex.

The vagotomy-induced attenuation or loss of the blood pressure component in the esophago-cardiovascular reflex cannot be attributed to the removal of vagal tone on heart. Our results show that the M-receptor antagonists, methscopolamine and scopolamine, did not significantly block the reflex. Moreover, there was no significant change in the MAP response to either noxious paw pinch or xylene administration after vagotomy. On the other hand, it was noted that the heart rate

response to noxious stimulation of the skin persisted after vagotomy, although bilateral vagotomy decreased the heart rate response to chemical or mechanical stimulation compared with unilateral vagotomy. These observations: 1) confirm the integrity of the central sites that mediate reflex cardiovascular responses to noxious stimulation following transection of the vagus nerve; and 2) strongly suggest that the sensory information triggering the esophago-cardiovascular reflex is conveyed to these sites by vagal, rather than spinal, nerves.

Topical administration of a very small amount (0.1-1.0 nmol) of DX or morphine to the solitary complex inhibited or even abolished the cardiovascular response to esophageal distension. This observation is consistent with the hypothesis that viscerosensory information in this reflex is transmitted to the CNS via vagal afferents because the latter are known to terminate in the NTS (Sumal *et al.*, 1983; Altschuler *et al.*, 1989). Furthermore, the results suggest that opioid and α_2 adrenoceptors in the NTS may play an important role in regulating esophageal peristalsis.

Physiological/pharmacological properties and central pathways of the esophago-cardiovascular reflex

Distal esophageal distension has been demonstrated to cause rhythmic esophageal peristalsis in urethane-anaesthetized rats (Lu and Bieger, 1992). At distension pressures in the range of 50 mmHg, the cardiovascular response was

accompanied by rhythmic peristalsis, and a fluctuation in blood pressure was evident which was tightly coupled to esophageal peristalsis. Esophageal peristalsis thus appears to modulate the esophago-cardiovascular reflex, presumably by augmenting the distension-evoked afferent discharge of intramural mechanoreceptors. This suggestion is consistent with the observation in sheep that esophageal mechanoreceptors are activated when the esophagus contracts (Falempin *et al.*, 1978).

On the other hand, at the stimulus intensity needed to elicit a maximal cardiovascular response, esophageal reflex peristalsis may have been inhibited, as evidenced by the absence of a superimposed rhythmic wave pattern in blood pressure. Conversely, at reduced stimulus strengths appropriate for eliciting reflex peristalsis, a rise in blood pressure was not evident until the first esophageal contraction occurred. The threshold of esophageal distension pressure for esophageal peristalsis is similar to the threshold for cardiovascular responses. However, the threshold for the unmodulated cardiovascular response was 5-10 times higher than that for esophageal peristalsis.

The pressor reflex *per se* was unequivocally dissociated from esophageal peristalsis since it persisted after esophageal paralysis induced by concurrent administration of pancuronium, scopolamine and methscopolamine. It was not possible to determine a change, if any, in the cardioaccelerator component of the esophago-cardiovascular reflex, owing to its occlusion by anticholinergic-induced

tachycardia. However, these data indicate that the heart rate and blood pressure responses to esophageal distension are independent events.

The effect of capsaicin on nociceptive reflexes and the esophago-cardiovascular reflex

Esophageal distension-evoked cardiovascular responses were significantly inhibited in neonatal capsaicin-treated rats, suggesting that capsaicin-sensitive fibers play an important role in mediating this reflex. Our observations, showing an increase in mechanical and thermal nociceptive threshold in adult rats and the depletion of capsaicin-sensitive substance P- and CGRP-immunoreactive fibers in the esophagus, confirmed the neurotoxic effect of capsaicin in these animals.

The NTS neuropil, vagal trunk, and nodose ganglia contain substance P, CGRP, vasoactive intestinal polypeptide (VIP), somatostatin (SST), cholecystokinin (CCK) and other peptides (for review see Van Giersbergen *et al.*, 1992). Substance P immunoreactive terminals also exist in the intermediolateral cell column (IML) (Takano and Loewy, 1985). Treatment of newborn rats with capsaicin resulted in substantial degeneration of unmyelinated primary sensory fibers (for review see Lynn, 1990) and depletion of neuropeptides from NTS, spinal dorsal horn, nodose ganglia, and vagus nerves (for review see Buck and Burk, 1986). However, there are very few CGRP- and Substance P-immunoreactive fibers in the subnucleus centralis of the solitary tract (NTS_c) (Cunningham and Sawchenko,

1990) where esophageal afferents terminate. This argues against CGRP and/or substance P playing a role in mechanoreception. However, both SST and enkephalin (L-ENK) related peptides have been found in the esophageal sensory portion of the nucleus of the solitary tract (NTS) (Cunningham and Sawchenko, 1989, 1990). SST has been shown to play a role in esophagomotor control (Wang *et al.*, 1991). The depletion of SST and L-ENK from NTS_c caused by neonatal capsaicin may contribute to the partial attenuation of the esophago-cardiovascular reflex although cardiopulmonary or baroreceptor function are reportedly unchanged (Meller *et al.*, 1991). The depletion of substance P in the IML may also contribute to the change.

As argued above, spinal afferents may not contribute to the esophago-cardiovascular reflex. Accordingly, the attenuation of the esophago-cardiovascular reflex by neonatal capsaicin may be attributable to the depletion of neuropeptides in the vagus nerves, NTS, IML, and other parts of the CNS. According to Green and Dockray (1987), both substance P and CGRP occur in a relatively small percentage of esophageal sensory neurons in the nodose ganglia. The few CGRP- and substance P-immunoreactive fibers within the NTS_c could arise from these nodosal afferents. Since depletion of substance P and CGRP alone from the NTS_c and vagal afferents resulted only in a partial attenuation of the esophago-cardiovascular reflex in neonatal capsaicin-treated rats, other afferent mediators are likely to be involved. The neurotransmitter(s) of esophageal vagal afferents to the NTS_c is/are

largely unknown and the function of capsaicin-sensitive fibers in the vagus nerve is still poorly understood. Nevertheless, vagal afferents are the most likely target, since capsaicin effects on the central neuropeptide neurons would not be lasting or very pronounced.

α_2 Adrenoceptors in the NTS and the esophago-cardiovascular reflex

The vagal afferents enter the solitary tract and terminate primarily in the NTS, with some continuing into the medial reticular formation of medulla, the cerebellum, the nucleus cuneatus, area postrema, and elsewhere (Rutecki, 1990). According to Altschuler *et al.* (1989), vagal esophageal afferents project exclusively to the subnucleus centralis of the NTS (NTS_c).

Sumal *et al.* (1983) demonstrated that sensory afferents from the nodose ganglion terminate mainly in the medial and caudal portions of the NTS containing catecholaminergic neurons. They also showed that dendrites of catecholaminergic neurons receive direct synaptic input from vagal afferents. These synapses of vagal afferents on catecholaminergic dendrites constitute a basis for the peripheral modulation of diverse autonomic functions which may be elicited by secondary synaptic interactions with the NTS or in projections to other regions of the CNS. The α_2 -adrenoceptors, presumably in NTS, are believed to mediate the hypotensive effects of central acting α_2 -adrenoceptor agonists (e.g., De Jong and Nijkamp, 1976). Our results, in which the topical administration of DX to the NTS inhibited

the esophago-cardiovascular reflex, suggested that α_2 -adrenoceptors on catecholaminergic neurons in NTS might be involved in the regulation of the esophago-cardiovascular reflex. The neurotransmitters in vagal afferents terminating on catecholaminergic dendrites remain unknown. Sumal *et al.* (1983) speculated that one of these neurotransmitters might be substance P. If correct, this would at least partially explain the attenuation of the esophago-cardiovascular reflex in neonatal capsaicin-treated rats.

Opioid receptors in the NTS and the esophago-cardiovascular reflex

Opioid receptors are present on the vagus nerves (Zarbin *et al.*, 1990) and the NTS (Atweh and Kuhar, 1977). They are believed to be involved in the regulation of cardiovascular activity, although in the resting condition, endogenous opioid peptides appear to be inactive. For example, the systemic or NTS topical administration of the opioid receptor antagonist, naloxone, does not significantly change basal blood pressure and heart rate (Petty De Long, 1982). While the i.v. administration of morphine decreases blood pressure and heart rate, these effects are secondary to the release of histamine (primarily from mast cells) and to increased parasympathetic or decreased sympathetic tone (Evans *et al.*, 1952, Feldberg and Paton, 1951). Recent studies by Rangach and his colleagues (1991) have shown that the bradycardia induced by i.v. morphine was attenuated by cervical vagotomy. Transection of the vagus nerve had no effect on the depressor

response produced by lower doses of morphine (≤ 0.5 mg/kg), whereas at higher doses, a pressor response (1.0 mg/kg) or an initial pressor response followed by a depressor response (2.5 mg/kg) was observed. These data suggest that the hypotension and bradycardia induced by i.v. morphine depend, in part, on the integrity of the vagus nerves.

The present investigation shows that the topical administration of morphine to the NTS produced a dose-dependent, naloxone-reversible inhibition of the cardiovascular responses triggered by esophageal distension. These results indicate that opioid receptors in the NTS not only contribute to the modulation of cardiovascular activity, but also play an important role in the regulation of afferent input from esophageal vagal fibers. As morphine readily crosses the blood brain barrier, its inhibitory effects on the esophago-cardiovascular reflex may be mediated, at least in part, by opioid receptors in the NTS. Another possible explanation is that morphine acted on opioid receptors on the vagus nerves, thereby inhibiting the vagal afferent transmission.

NTS efferents to the RVLM

The NTS receives vagal afferents and projects efferents to many different parts in the forebrain, lower brain stem, and spinal cord (Van Giersbergen *et al*, 1992). In view of the prominent role of the RVLM in cardiovascular control, the question arises if this region receives input from that part of NTS known to be

innervated by esophageal vagal afferents, namely the NTS_c.

Anatomically, the RVLM neurons project their axons dorsally and caudally in the lateral column of the spinal cord to the IML (Ganong, 1993b). The neurons known as the C1 adrenergic cell group, whose cell bodies are located near the pial surface of the medulla in the rostral ventrolateral reticular nucleus, provide tonic excitation to sympathetic preganglionic neurons in the IML. This input is necessary for the maintenance of resting sympathetic tone, and serves as the final common reticulospinal pathway mediating the baroreceptor and other somato-sympathetic reflexes (Morrison *et al.*, 1988).

Retrograde tracer experiments by Ross *et al.* (1985) showed that the RVLM receives direct projections from the medial, ventral, intermediate, and interstitial portions of the ventrolateral subnuclei of the ipsilateral NTS. In the same study, the RVLM was also claimed to receive afferent inputs from the NTS_c, where the esophageal vagal afferents terminate (Altschuler *et al.*, 1989). The existence and possible role of NTS_c efferents to the RVLM in the esophago-cardiovascular reflex remain to be verified. Neurons in the region of the RVLM innervated by the NTS are adrenergic (Ross *et al.*, 1985). However, the identity of the NTS neurons that connect the vagal afferents and the RVLM remains unclear. The caudal part of NTS, together with the commissural nucleus, is known to form the primary medullary center for a multisynaptic cardiovascular reflex arc. Lesions of these areas result in acute fulminating hypertension, while electrical stimulation yields the opposite

effect (Bystrzycka and Nail, 1985). The A2 cell group of catecholamine, principally norepinephrine, neurons in the caudal commissural and medial subnuclei in NTS is known to be involved in central cardiovascular regulation (Zandberg *et al.*, 1979). It may provide the internuncial neurons that modulate the efferent limb of the esophago-cardiovascular reflex, although this would require an additional inhibitory interneuron in the reflex arc.

In addition to projecting to the RVLM, some NTS neurons also project directly to the IML (Loewy and Burton, 1978, Tucker and Saper, 1985). However, there are no data to confirm that any of these projections originate in the NTS. Moreover, the function of these projections remains unclear. Further investigation will be required to determine the role, if any, of these projections in the esophago-cardiovascular reflex.

The efferent limb of the esophago-cardiovascular reflex

The C1 group of cells in the RVLM projects directly to the IML in the spinal cord (Milner *et al.*, 1989). Phenylethanolamine-N-methyltransferase, the enzyme responsible for the formation of epinephrine from its precursor, norepinephrine, is localized in, and epinephrine is released from neurons originating in the C1 adrenergic cell group. Whether spinal epinephrine is sympathoexcitatory or inhibitory remains uncertain (Morrison *et al.*, 1988).

The i.t. administration of DX, a selective α_2 -adrenoceptor agonist, into the thoracic spinal cord significantly inhibited the cardiovascular response to esophageal distension. In contrast, the i.v. or i.t. lumbar administration of the same dose of DX had no significant effect on the reflex, despite a decrease in *basal* MAP and heart rate. Although DX has been shown to be antinociceptive following injection into the lumbar subarachnoid space of rats (Fisher *et al.*, 1990), our data suggest that i.t. DX inhibited the cell bodies of preganglionic sympathetic neurons located in the IML of the thoracic spinal cord, and not the central terminals of spinal afferent fibers from the esophagus. The failure of i.t. morphine to block the esophago-cardiovascular reflex is consistent with this conclusion.

Preganglionic sympathetic neurons in the IML project to sympathetic ganglia in the periphery and to the adrenal medulla where they release acetylcholine. This, in turn, evokes the release of norepinephrine from postganglionic neurons, and norepinephrine, epinephrine and dopamine from the adrenal medulla that act on the heart and arteriolar beds to increase both blood pressure and heart rate. Using another model of visceral nociception, Ness and Gebhart (1988) demonstrated that adrenal demedullation significantly decreased tachycardia, but not the pressor response, to colorectal distension. They also showed that these cardiovascular responses were differentially blocked by the α -adrenoceptor antagonist, phentolamine, and the β -adrenoceptor antagonist, propranolol. Their results, and the data from the present experiments suggest that the final step in visceral

distension-evoked cardiovascular responses are mediated by the reflex activation of sympathetic efferents in the spinal cord.

Conclusions

The evidence obtained in this investigation supports the following conclusions: 1) sensory information in the esophagocardiovascular reflex is transmitted to the central nervous system via vagal afferents, (2) capsaicin-sensitive vagal afferents from the esophagus may constitute a subpopulation of neurons in the afferent limb of this reflex, (3) the efferent limb of this reflex is comprised of sympathetic cardioaccelerator- and vasoconstrictor-preganglionic neurons located in the intermediolateral cell column of thoracic spinal cord, (4) the internuncial connections are made up of bulbo-spinal pathways probably originating in the rostral ventrolateral medulla (RVLM) which, in turn, receive input from the NTS, (5) the pressor reflex is dissociated from esophageal peristalsis, (6) internuncial transmission at the level of the NTS neurons is blocked by both morphine and dexmedetomidine, (7) transmission at the level of the sympathetic preganglionic neurons is sensitive to dexmedetomidine, but resistant to morphine, (8) the failure of spinal morphine to affect the pressor reflex argues against the involvement of nociceptive dorsal root ganglia (DRG) afferents, (9) the spinal and supraspinal sites of action of dexmedetomidine support a role of bulbo-spinal adrenergic neurons in this reflex, and (10) further studies of the NTS interneurons which connect the vagal

afferent and sympathetic efferent limbs are required. Figure 18 presents the postulated organization of the esophago-cardiovascular reflex circuitry.

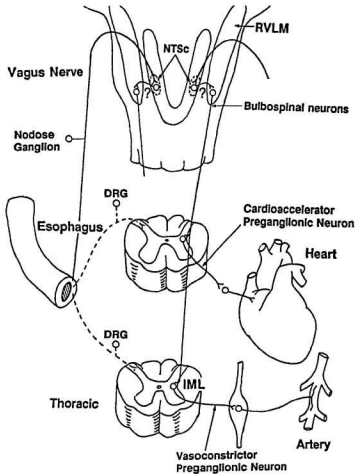


Figure 18. The postulated esophago-cardiovascular reflex arc. For clarity, the afferent limb of the esophagocardiovascular reflex is shown on the left and the efferent limb is shown on the right half of the diagram. DRG = Dorsal root ganglion; IML = intermediolateral cell column; NTS_c = Subnucleus centralis of the nucleus tractus solitarius; RVLM = rostromedial lateral medulla

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