

**NEUROTRANSMITTER CONTENT OF PARASYMPATHETIC AND SYMPATHETIC NEURONS SUPPLYING THE AIRWAYS OF THE RAT.** J.J. Bowden and I.L. Gibbins, Departments of Human Physiology and Anatomy and Histology, Centre for Neuroscience, Flinders University of South Australia, Australia.

The airways of the rat differ functionally from those of many other mammalian species in that they appear to lack a non-adrenergic non-cholinergic inhibitory neural input to airway smooth muscle. We have used a combination of immunohistochemistry and retrograde neuronal tracing to determine the neurotransmitter content of parasympathetic and sympathetic neurons supplying the airways of the rat. Three populations of parasympathetic neurons were identified; those containing immunoreactivity (IR) to neuropeptide Y (NPY), (70% of cell bodies in intrinsic ganglia, n = 4 animals), those containing IR to both vasoactive intestinal peptide (VIP) and NPY, (24% of cell bodies, n = 4 animals) and those containing IR to VIP and the proenkephalin derivative MEAGL, but not NPY-IR. IR to substance P, calcitonin gene related peptide, galanin or tyrosine hydroxylase (TH, a marker for noradrenergic neurons) was absent from intrinsic airway neurons. NPY-IR nerve fibres were abundant in airway smooth muscle; rarely did such fibres contain VIP-IR or TH-IR (n = 8 animals). Nerve fibres containing both VIP-IR and NPY-IR supplied tracheal and bronchial blood vessels and were also present within the epithelium. After injection of the neuronal tracer Dil into the posterior tracheal wall, retrogradely labelled nerve cell bodies were identified in the stellate ganglion (1134 cells, n = 4 animals). On the basis of previous immunohistochemical studies and combined immunohistochemistry and retrograde labelling of nerve cell bodies, 3 populations of sympathetic neurons supplying the airways were identified; those containing VIP-IR (but neither NPY-IR nor TH-IR, 15% of labelled cells), those containing NPY-IR and TH-IR (but not VIP-IR, 59%) and those containing TH-IR (but neither VIP-IR nor NPY-IR, 26%). Nerve fibres containing VIP-IR (but not NPY-IR) were present within the subepithelial layer, while fibres containing both TH-IR and NPY-IR supplied tracheal and bronchial blood vessels. These results demonstrate that neurotransmitters may coexist in specific populations of neurons supplying specific targets within the airways, and that the same neurotransmitter (e.g. VIP or NPY) may be present in both parasympathetic and sympathetic neurons supplying the airways of this species. The effects of NPY on cholinergic or noradrenergic neurotransmission within the airways, and the role of VIP-IR sympathetic neurons will require further investigation.

Supported by the NHMRC of Australia.

**COLOCALISATION OF NEUROTRANSMITTERS IN AUTONOMIC NEURONS SUPPLYING THE RESPIRATORY TRACT OF VARIOUS SPECIES, INCLUDING HUMANS.**

J.J. Bowden and I.L. Gibbins, Departments of Human Physiology and Anatomy and Histology, Centre for Neuroscience, Flinders University of South Australia, Australia.

Colocalisation of both classical and neuropeptide neurotransmitters has been described in autonomic and sensory neurons supplying a range of tissues. Such colocalisation may be specific for a population of neurons supplying a particular target, and may vary between species. We have used double-labelling immunohistochemistry directed against a range of neuropeptide neurotransmitters and synthetic enzymes to identify patterns of coexistence in autonomic neurons supplying the airways in a range of species. Immunoreactivity to vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), substance P (SP), galanin (GAL), and tyrosine hydroxylase (TH, a marker for presumed noradrenergic sympathetic neurons) was identified in sections of bronchi and trachea and patterns of coexistence determined as outlined below.

Species	Airway Smooth Muscle	Submucosal Glands	Blood Vessels	Intrinsic Airway Ganglia
Human (n=12)	VIP/NPY* VIP/GAL* TH/NPY*	VIP TH	TH/NPY TH	VIP/NPY VIP/GAL
Cats (n=12)	VIP VIP/SP TH/NPY	VIP/SP VIP/GAL TH/NPY	VIP TH/GAL TH/NPY	VIP VIP/SP VIP/GAL
Sheep (n=6)	VIP/NPY/GAL TH/NPY	-	TH/NPY VIP TH	VIP/NPY/GAL
Pigs (n=5)	VIP/NPY/GAL TH/NPY	-	VIP/GAL TH/NPY TH	VIP/NPY/GAL
Rabbits (n=6)	VIP/NPY NPY	-	TH/VIP TH/NPY	VIP/NPY NPY

\* Indicates populations of neurons which are rare within target tissue

These results demonstrate species specific and target specific patterns of coexistence of neurotransmitters supplying the airways. An understanding of such differences is critical in the design and interpretation of pharmacological studies of the effects of various neurotransmitters on airway function.

Supported by the NHMRC of Australia.

**A SEMI-QUANTITATIVE COMPARISON OF VASOACTIVE INTESTINAL PEPTIDE (VIP) IMMUNOREACTIVE NERVE FIBRE DENSITY IN NORMAL AND ASTHMATIC TRACHEAL AND LARGE BRONCHIAL SMOOTH MUSCLE.** T.R. Bai, J. Hards and P.D. Paré. UBC Pulmonary Research Laboratory, St. Paul's Hospital, Vancouver, B.C. V6Z 1Y6.

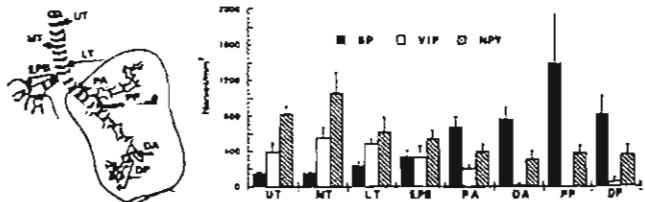
A decrease in neuronal VIP content within airway walls has been proposed to contribute to airway hyperresponsiveness in asthma and there has been one report in support of this hypothesis (N Engl J Med. 320; 1244-8). In this pilot study, a semi-quantitative analysis of VIP and protein gene product 9.5 (PGP9.5 - a non specific neuronal marker) immunoreactive nerve fibre density has been performed on 8 µm cryostat sections of trachea and main bronchus, fixed in Zamboni's medium, from three asthmatic and three normal subjects. Tissues were obtained at autopsy following fatal asthma attacks or sudden non-pulmonary death. Following application of primary antisera to VIP or PGP9.5 on serial sections, a secondary fluorescein -isothiocyanate antiserum was applied. Twelve sections from each subject were analyzed. All immunofluorescent nerve fibres within randomly selected smooth muscle bundles were photographed on slide film which was then projected onto a digitizing tablet. A blinded observer then digitized the total length of nerve fibre and smooth muscle area within each photograph so that density (fibre length in mm/mm<sup>2</sup> of muscle) could be calculated. VIP-immunoreactive nerve fibres were visualized in all asthmatics. No systematic differences were detected in PGP9.5 or VIP nerve fibre density between asthmatic and normal airways. Overall nerve density ranged from 2.0 to 50.7 mm/mm<sup>2</sup> and VIP immunoreactivity from 0.56 to 1.18 mm/mm<sup>2</sup>. Although further analysis is pending of relative nerve densities between asthmatics and normals, the results do not confirm an absolute loss of VIP neuronal immunoreactivity in asthma.

Supported by the B.C. Health Research Foundation.

20044911

**QUANTITATIVE MORPHOMETRIC ANALYSIS OF SUBSTANCE P, VASOACTIVE INTESTINAL PEPTIDE, AND NEUROPEPTIDE Y IN GUINEA PIG AIRWAY.** Linda Manley and Richard D. Dey, Department of Anatomy, West Virginia University, Morgantown, WV 26506.

As putative neurotransmitters of nonadrenergic, noncholinergic (NANC) nerves, neuropeptides have various actions on airway smooth muscle. However, the magnitude of their effects depends on airway level and may be related to the density of innervation. This study quantifies the distribution of substance P (SP), vasoactive intestinal peptide (VIP), and neuropeptide Y (NPY), in smooth muscle of different airway levels. Guinea pig airways (n=4) from trachea through small bronchi were divided into eight levels: upper, middle, and lower trachea (UT, MT, LT), extrapulmonary bronchi (EXB), proximal (PA) and distal (DA) axial bronchi, and the pooled 2nd to 5th generations from the proximal (P2-5) and distal (D2-5) axial bronchi (see diagram). Sections from each level were immunocytochemically labeled for SP, VIP, and NPY. Density of innervation was determined by measuring the number of nerve fibers per mm<sup>2</sup> (n/mm<sup>2</sup>) of airway smooth muscle. The results are summarized in the following graph:



SP is present at all levels but highest in smaller airways. VIP is most abundant in the trachea and absent in smaller airways. NPY is present at all levels but highest in the trachea. Thus, the density of neuropeptide innervation is regionally heterogeneous for each neuropeptide. The distribution described is consistent with physiological studies on the actions of neuropeptides on airway smooth muscle at different levels. (Supported by NIOSH Cooperative Agreement #CCU306149 and NHLBI #K04 HLD2125).

AMERICAN REVIEW OF

# Respiratory Disease

SUPPLEMENT

April 1992

Volume 145

Number 4, Part 2

AMERICAN LUNG ASSOCIATION • AMERICAN THORACIC SOCIETY

ABSTRACTS

1992 International Conference

May 17-20, 1992 • Miami Beach, Florida

Contents .....	A3
Sunday, May 17 .....	A9
Monday, May 18 .....	A215
Tuesday, May 19 .....	A449
Wednesday, May 20 .....	A679
Index .....	A883

This special supplement of the *American Review of Respiratory Disease* contains abstracts of the scientific papers to be presented at the 1992 International Conference, which is sponsored by the American Lung Association and the American Thoracic Society. The abstracts appear in order of presentation, from Sunday, May 17 through Wednesday, May 20 and are identified by session code numbers. To assist in planning a personal schedule at the Conference, the time and place of each presentation is also provided.