MAO activities in rat and human whole brain homogenates (H) and microvessels (M) assayed with serotonin (5-HT) and phenylethylamine (PEA)

	V_{max} (nmoles/mg/h) 5-HT	PEA	5-HT:PEA
Rat brain, whole H (N = 4)	76.9 ± 14.5	37.0 ± 5.1	2.1
Rat brain, M-free H (N = 4)	60.1 ± 11.3	30.4 ± 8.8	2.0
Rat brain, M (N = 4)	63.8 ± 17.4	25.3 ± 8.1	2.5
Human frontal cortex, whole H (N = 3)	17.8 ± 2.3	14.6 ± 3.9	1.2
Human frontal cortex, M (N = 3)	21.7 ± 1.6	14.6 ± 4.0	1.5
Human frontal cortex, M[a]	16.3; 14.1	14.2; 11.3	1.2; 1.3
Human sensorimotor cortex, M[a]	62.0; 51.0; 17.1	14.0; 10.0; 4.7	4.4; 5.1; 3.6
Human thalamus, M[b]	11.8; 3.7; 35.7	5.6; 1.9; 20.4	2.0; 1.9; 1.8
Human hypothalamus, M[b]	12.2; 17.6; 21.0	30.1; 23.3; 25.0	0.4; 0.8; 0.8
Human cerebellum, M[a]	37.0; 56.8; 48.0; 28.0	4.6; 6.8; 5.0; 3.7	8.0; 8.4; 9.6; 7.5
Human frontal cortex, stillborn infant, M[a]	24.1	12.0	2.0

[a] Data represent individual values. [b] Data represent 3 sets; each set consists of tissues from 3 individuals.

procedure which microscopically gives us better separation and recovery than the 'sieving' procedure used by Lai et al.[9,10]. Similar results in enzyme activities were obtained for human cortex; no major difference in MAO type A and B activities was observed between whole brain homogenate and microvessels.

Processing of some human brain areas necessitated pooling of samples due to the small amounts available from different individuals. MAO activities and ratios from the same regions in these separate sets were found to be remarkably similar. This allows for a comparison of these sets among brain regions. In general, human MAO activities in microvessels are somewhat lower than those found for whole rat brain. Marked differences in MAO activities and ratios were found among the areas studied. The differences range from a 5-HT:PEA ratio of about 0.7 for hypothalamus to a ratio of about 8.0 for cerebellum. Thus, the distribution of both MAO activities in microvessels is not uniform throughout the brain but seems to be characteristic for a particular brain region. This is in contrast to the finding that MAO type A and B activities do not differ significantly in crude homogenates of different human brain regions[18]. Since microvessels constitute only a small fraction of the homogenates, it can be assumed that neuronal and glial MAO is more evenly distributed throughout the human brain wheras MAO activities in microvessels show a more uneven distribution. The latter finding is interesting since MAO is part of the 'enzymatic' blood-brain-barrier[8,19] and differences in blood vessel MAO activities could selectively regulate which amine would or would not cross the vessel wall and penetrate into a particular brain region.

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Bombesin, calcitonin and leu-enkephalin immunoreactivity in endocrine cells of human lung

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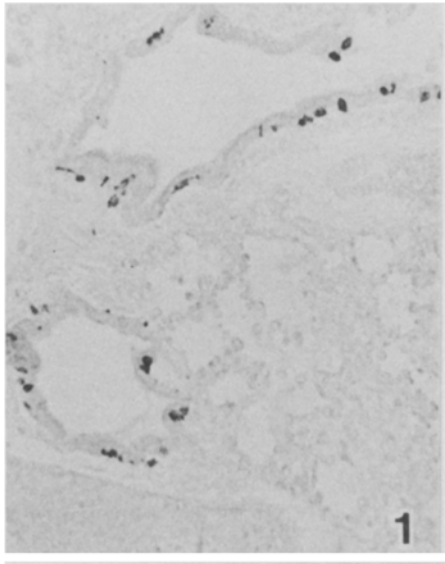
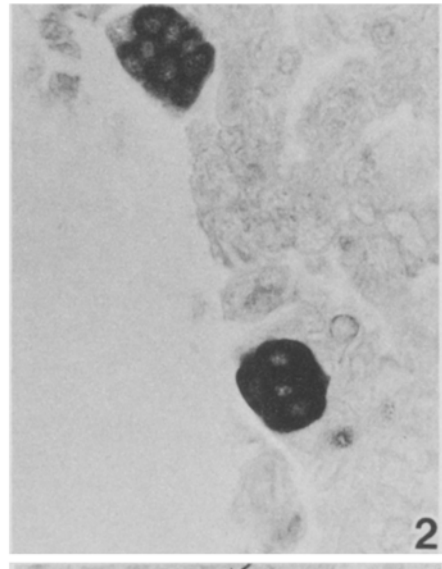
Summary. By immunohistochemistry, bombesin, calcitonin and leu-enkephalin was localized in endocrine cells of human lungs from various age groups. It is suggested that at least 3 different peptide containing endocrine cells may be present in human lung.

Over 40 years ago Feyrter described argyrophilic, clear cells (Helle Zellen) in bronchial epithelium and suggested that they may have an endocrine or paracrine function[3]. Subsequent studies confirmed the presence of these cells in fetal, newborn and adult lungs of human and various animal species[4–8]. These cells were found to be distributed singly, and in distinctive innervated corpuscles referred to as neuroepithelial bodies (NEB)[9]. Histochemical and ultrastructural studies have shown that these cells possess the capacity to take up amine precursors and contain neurosecretory granules - features characteristic of the system of polypeptide hormone producing APUD (amine precursor uptake and

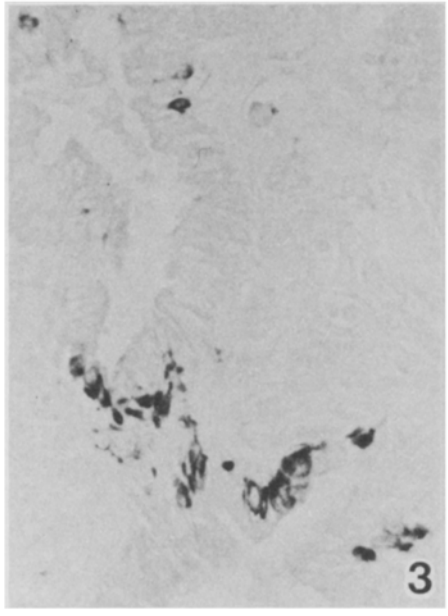
decarboxylation) cells[10]. By analogy with APUD cells elsewhere it has been postulated that lung endocrine cells may also produce polypeptide hormones[11,12]. Wharton et al. have recently reported occurrence of bombesin-like immunoreactivity in endocrine cells of human fetal and newborn lungs but not in older age groups[13]. Calcitonin-like immunoreactivity has been described in endocrine cells of human newborn lungs[14]. We report on IH localization of bombesin, calcitonin and leu-enkephalin-like immunoreactivity in endocrine cells of human lung from various age groups.

Materials and methods. Lung samples were studied from human fetuses, newborns, children and adults. Samples of lung from fetuses (12–20 weeks gestation) were obtained at therapeutic abortions, and from other age groups at autopsies (1–24 h after death) from patients without primary respiratory disease or from normal areas of surgically resected lungs. At least 5 different cases were included in each age group. The samples were fixed in Bouin's fluid or 10% neutral buffered formalin and embedded in paraffin. For IH studies indirect immunoperoxidase (IP) method, according to Nakane[15] and IP method of Sternberger[16] using the peroxidase-antiperoxidase (PAP) complex were employed. Paraffin sections cut at 6 µm were dewaxed, washed in phosphate buffered saline (pH 7.1), and incubated with peptide antisera for 18–24 h at 4 °C in a moist chamber. The following peptide antisera, raised in rabbits were used: anti-bombesin (L89) was a gift from Dr G. Dockray, University of Liverpool, U.K.; anti-bombesin (lot 24,109) from Immuno Nuclear Corp., USA; anti-human calcitonin (lot 860,188) from Calbiochem-Behring Corp., USA; anti-human calcitonin (lot 35,189) and anti-leucine-enkephalin (lot 47,169) from Immuno Nuclear Corp., USA. The peptide antisera were used in following dilutions: bombesin 1:400–1:5000; calcitonin 1:250–1:2000 and leu-enkephalin 1:400–1:1000. For IP method of Nakane, the sections were incubated with peroxidase conjugated swine-antirabbit antiserum (Dako, Denmark) in 1:10 dilution, followed by incubation with 3, 3′-diamino benzidine (Sigma, St. Louis, USA). For PAP method, following incubation with the primary peptide antibodies, the sections were treated with goat-antirabbit IgG (Cappel Lab. USA) in 1:20 dilution and then with PAP complex (Dako, Denmark) diluted 1:50. Immunostaining for the 3 peptides was performed on consecutive sections from the same tissue blocks. As controls, nonimmune serum and peptide antiserum previously absorbed with respective pure





Figures 1 and 2. Sections of lung from a 5-year-old child incubated with antiserum against bombesin. Figure 1. Numerous strongly immunoreactive cells scattered within the epithelium of small bronchioles. ×90. Figure 2. Higher magnification from the same section shows 2 bombesin immunoreactive neuroepithelial bodies in an alveolar duct. ×400.



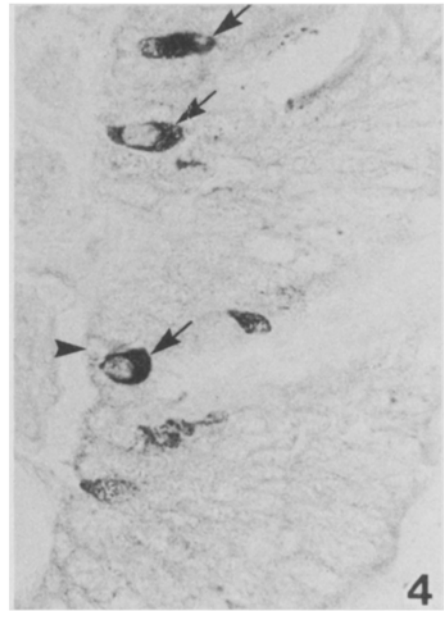


Figure 3. Single and groups of cells with calcitonin-like immunoreactivity in a peripheral airway of lung from a 1-month-old infant. ×225. Figure 4. Several single cells with leu-enkephalin-like immunoreactivity in the cytoplasm (arrows), located close to the bronchiolar basement membrane (arrowhead). Sections of lung from a 15-year-old patient. ×400.

peptide antigen (bombesin 2.5 μg/ml, calcitonin 25 μg/ml and leu-enkephalin 50 μg/ml) were used. These and other pure peptides (VIP, substance P, somatostatin), used in tests for cross-reactivity, were obtained from Peninsula Lab., USA.

Results. Immunostaining for the 3 peptides was identical using either of the 2 IP methods. The figures shown are from sections stained by the IP method of Nakane. The sections of lung incubated with either bombesin antisera (diluted up to 1:5000) showed numerous strongly immunoreactive cells scattered within the airway epithelium (fig. 1). Bombesin immunoreactive cells were found in lung samples from all age groups examined. In fetal lungs, bombesin immunoreactive cells were found at all levels of the developing bronchial tree; whereas in postnatal and adult lung they were concentrated mostly in peripheral airways. Single pyramidal or flask shaped cells possessed thin apical or lateral cytoplasmic processes and grouped immunoreactive cells, forming NEB were composed of 5–10 closely packed cells (fig. 2). In consecutive sections incubated with either calcitonin anti-sera (diluted up to 1:2000) fewer cells were stained but their distribution was similar to the bombesin cells (fig. 3). Single cells with calcitonin-like immunoreactivity were found at all levels of the bronchial tree in both fetal and postnatal lungs but only occasional NEB showed calcitonin-like immunoreactivity. Calcitonin immunoreactive cells appeared more numerous in neonatal and child lungs compared to fetal and adult lungs. Leu-enkephalin-like immunoreactivity (using antiserum diluted up to 1:1000) was detected in only a few single cells scattered within the epithelium of peripheral airways (fig. 4). Cells with leu-enkephalin-like immunoreactivity were identified in lung samples from all age groups, but were the least numerous of the 3 peptides tested. NEB cells were negative for leu-enkephalin staining. The specificity of peptide localization was confirmed for each antiserum used. The immunoreactivity was abolished by preabsorption of primary antisera with respective pure peptide antigen. The positive immunoreactivity was not affected when the antisera were absorbed with dissimilar or other brain-gut peptides (VIP, substance P, somatostatin). In control sections incubated with non-immune serum, no immunoreactivity was detected.

Discussion. The overall appearance, localization and distribution of immunoreactive cells in pulmonary epithelium corresponds to argyrophilic, amine and neurosecretory granule containing cells described in previous studies[4–9]. The identification of immunoreactivity to three different peptides (bombesin, calcitonin, leu-enkephalin) raises the question whether each peptide is localized in a specific type of lung endocrine cells or more than one peptide may be present in the same cell. By electron microscopy, 3 ultrastructurally distinct types of endocrine cells have been identified in human fetal lungs[6,7]. However in adult lung, only 1 type of endocrine cell was found[18]. This discrepancy could be due to the scarcity of these cells in adult lung or may represent developmental change. Our finding of different frequency and distribution of peptide immunoreactive cells in the lung, suggest that the three peptides are localized in different types of endocrine cells and that they persist throughout life. In this respect the endocrine cells in lung may be analogous to similar cells in the GI tract, where several cell types have been shown to contain different hormones[18]. For definitive identification and localization of each peptide in a specific type(s) of lung endocrine cells, studies using immunocytochemistry at EM level are required. Further studies are needed to confirm that the peptides identified in lung endocrine cells by immunocytochemistry, are being synthesized by these cells. The exact role of peptides in lung endocrine cells is presently unknown. However, the finding of polypeptide hormones in the lung has important implications for pulmonary physiology and pathophysiology. Bombesin appears to be the most abundant peptide in the human lung. Its presence in fetal, newborn and adult lungs suggest that it may play an important role during neonatal adaptation as well as postnatally. Bombesin has been identified in various mammalian tissues including the GI tract and the brain[19]. Bombesin has a wide spectrum of biological activities[20] with possible roles as a local or systemic hormone and a neurotransmitter[19]. Although this peptide has been reported to have powerful broncho-constrictor effect in the guinea-pig[21], its precise function in the lung remains to be elucidated. The presence of calcitonin-like immunoreactivity in pulmonary endocrine cells suggest that the lung may be an extra-thyroidal source of this hormone. In fact, calcitonin has been detected in humans and monkeys following total thyroidectomy[22]. The presence of calcitonin in normal lung may also explain the production of this peptide by certain lung neoplasms arising from these cells[23]. Cells with leu-enkephalin-like immunoreactivity were found to be the least frequent and were confined to single occurring pulmonary endocrine cells only. Leu-enkephalin belongs to a group of opioid peptides found in both endocrine cells and nerves in the GI tract, and in various regions of the brain[24]. The function of this peptide in the peripheral neuroendocrine cells is unknown. Clearly, more studies are indicated to define the function of lung endocrine cells and their peptide products.

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