Serotonin, CGRP, Calcitonin, CCK, Somatostatin and VIP in the Endocrine Cells of Developing Rat Lung

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SUMMARY

Immunoreactivity of serotonin and some regulatory peptides (calcitonin gene related peptide (CGRP), calcitonin, cholecystokinin (CCK), somatostatin) has been demonstrated in the endocrine cells of developing lung by the peroxidase anti-peroxidase method in rat. Immunocytochemistry revealed higher density of pulmonary endocrine cells containing serotonin and CGRP in foetal and early neonatal periods than in the lungs of older rats. Serotonin positive cells were mainly located within the bronchial epithelium and in alveolar sacs, whereas the localization of CGRP positive cells was essentially restricted to alveolar sacs. The calcitonin and somatostatin-containing cells were scarcely observed whatever the developmental stages examined in bronchi, bronchioles and in alveolar sacs. The CCK expression was weak and exclusively found in alveolar sacs, and remained constant from foetus to adult stages. VIP immunoreactivity was never detected during lung development in rat. These results suggest that serotonin and CGRP would be potent mediators involved in the lung ontogeny and in neonatal adaptation to air breathing.

Keywords : Serotonin - regulatory peptides - endocrine cell - development - lung - rat.

RÉSUMÉ

La production de sérotonine, de CGRP, de calcitonine, de cholécystokinine, de somatostatine et de VIP par des cellules endocrines durant le développement du poumon chez le rat. Par A. BAYRAKDAR et B. GENÇER TARAKÇI.

La production de sérotonine et de différents peptides (calcitonine, peptide associé au gène de la calcitonine (CGRP), cholécystokinine (CCK), et somatostatine) par des cellules endocrines a été démontrée dans les poumons en cours de développement chez le rat par immunohistochimie (método péroxydase-antipéroxydase). Les cellules endocrines pulmonaires contenant de la sérotonine ou du CGRP ont été plus fréquemment observées durant la période fœtale et le début de la période néonatale que dans les périodes ultérieures. Les cellules exprimant la calcitonine ont été principalement localisées dans l’épithélium bronchique et dans les alvéoles, tandis que les cellules positives pour le CGRP ont été observées essentiellement dans les alvéoles. Les cellules contenant la calcitonine et la somatostatine situées dans les bronches, les bronchioles et les alvéoles ont été détectées avec une faible densité quelle que soit le stade de développement considéré. L’expression de la CCK associée exclusivement aux alvéoles est également restée constamment faible du stade fœtal au stade adulte. Aucune activité envers le VIP n’a été décelée durant le développement pulmonaire chez le rat. Ces résultats suggèrent que la sérotonine et le CGRP seraient des médiateurs importants impliqués dans l’ontogenèse pulmonaire et dans l’adaptation du nouveau-né à la respiration aérienne.


Introduction

The occurrence of endocrine cells within the epithelium of the respiratory tract of foetal and adult mammals has been evidenced by many investigators. The history of endocrine cells dates back to 1938. Since FEYRTER [17] first suggested in 1938 an endocrine role for the “clear cells” of the lung, investigators have sought to identify hormones or their precursors in pulmonary epithelial cells. This cell type is now classified as the pulmonary neuroendocrine cells (PNECs) [29].

The PNEC system is composed of solitary cells and PNEC clusters, neuroepithelial bodies (NEBs), widely distributed in the airway mucosa of mammalian lung [1, 12, 29, 37]. The solitary PNECs are found throughout the tracheobronchial epithelium, while the NEBs are found only in the intrapulmonary airways [10, 20, 38]. NEB together with solitary PNECs constitute a multifunctional PNEC system with potential roles during lung development, neonatal adaptation and in a variety of prenatal pulmonary disorders [12]. Because NEBs are prominent in foetal/neonatal lungs, their role may be particularly important during lung development and neonatal adaptation [11, 13, 29]. Although the precise role of the PNEC system is unknown, recent studies indicate that NEBs may function as airway O2 sensors [29, 42]. The function of PNEC/NEB is modulated via amine and neuropeptide mediators [18]. The principal amine produced by PNEC/NEB is serotonin (5-hydroxytryptamine, 5-HT) which has been evidenced in PNEC system of many species [2, 9, 14, 20, 24, 33].

Several studies have reported expression of serotonin and some neuropeptides in PNEC system of various species during ontogeny [22, 26, 30, 31]. However, little information concerning these mediators during the intermediate period of rat life (between birth and the development of the mature lung) is available. Thus, the present study investigated serotonin and some neuropeptides occurrence in the pulmonary endocrine cells of rat during different development stages by immunohistochemistry.
Materials and Methods

Twenty day old Wistar rat foetuses, new born, 5 to 30 days old and 60 day old rats were studied (at least 4 of each age). Rats were housed under controlled conditions of temperature and light. Pelleted food and water were supplied ad libitum. After euthanasia with ether, lungs were removed and fixed in 10% neutral buffered formaldehyde, for 24 hour. They were then dehydrated through graded ethanol and embedded in paraffin. 5µm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by using the peroxidase-antiperoxidase (PAP) method. Blocking of endogenous peroxidase was carried out with 0.0025% hydrogen peroxide in methanol for 10 minutes [35]. In order to block unspecific binding, an incubation with (1:10) normal goat serum in 0.1M phosphate buffered saline (PBS), pH 7.2 was performed. Sections were incubated for 16-20 hours at 4 °C with rabbit IgG antibodies against serotonin (Zymed Lab., 18.0077), calcitonin gene-related peptide (Chemicon, AB5920), calcitonin (Zymed Lab.,18.0012), cholecystokinin (Chemicon, AB2973), somatostatin (Chemicon, AB1976) or vasoactive intestinal polypeptide (Chemicon, AB982). Antibodies were diluted to 1:100, 1:500, 1:100, 1:200, 1:50 and 1:50 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in goat anti-rabbit IgG (DAKO, Z0421), followed by rabbit peroxidase anti-peroxidase complex (Zymed lab, 61.2003), both at dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation and finally immersed in GDN (Glucose oxidase-DAB-Nickel) [32] substrate for 10 minutes. After washing in distilled water and counterstaining with eosin, sections were dehydrated and cover slips mounted with aqueous permanent mounting medium. The specificity of each immunohistochemical reaction was determined as recommended by STERNBERGER [34] by using the specific antiserum preincubated with its corresponding antigen. Sections were examined with light microscope and photographs were taken.

Results

Serotonin, calcitonin gene-related peptide (CGRP), calcitonin, cholecystokinin (CCK) and somatostatin positive pulmonary endocrine cells were identified in all rats whereas VIP was never detected. The distribution and the relative frequency of these imunoreactive cells in developing lungs of rats are shown in Table I. Serotonin and CGRP endocrine cells were more frequently encountered in foetuses, newborns and in very young animals (5 day old) than in older rats (more than 10 days old) and the relative densities of serotonin and CGRP positive cells were maximal in 5 day old rats and in newborn / 5 day old rats respectively. Thereafter, the numbers of these cells remained relatively constant in young and in adult rats. On the other hand, calcitonin, CCK and somatostatin endocrine cells were observed in the lungs of foetuses, newborns or young animals (5 to 30 day old) with a low frequency, whereas these pulmonary cells appeared more rarely in adult stage.

In all stages examined serotonin immunoreactive endocrine cells were usually located solitarily or formed clusters in the bronchi and bronchioles (Figure 1). Some serotonin positive cells were extended along the basement membrane. Immunoreactive endocrine cells were also found in the alveolar sacs (Figure 2).

![FIGURE 1. — Serotonin-immunoreactive neuroendocrine cell (arrow head) and neuroepithelial body (arrows) in the bronchi of 5 day old rat lung. 200x.](image1)

![FIGURE 2. — Neuroepithelial body containing serotonin in the alveolar sac of 20 day old rat lung (arrow). 200x.](image2)
CGRP immunoreactive endocrine cells were found mainly in a NEB (neuroepithelial body) form in alveolar sacs and in intrapulmonary airways (Figure 3). Solitary neuroepithelial cells (NECs) also showed immunoreactivity (Figure 4).

Calcitonin immunoreactivity was generally detected in solitary neuroepithelial cells located in bronchi and bronchioles (Figures 5 and 6), whereas positive NEB were only found in alveolar sacs. CCK immunoreactive cells were usually observed in NEB form located in alveolar sacs (Figure 7). Somatostatin immunoreactive endocrine cells were seen in NEB and solitary NEC forms in bronchi, bronchioles and alveolar sacs (Figures 8 and 9).

**Discussion**

Several studies have been already published on the quantification of PNEC system in lungs of guinea-pig, rabbit, cat, rat, hamster and man [9, 11, 36, 38]. A great heterogeneity...
for peptide detection and for endocrine cell distribution was encountered between reports in literature due to the differences in staining methods, inherent technical variables and interspecies differences. But the highest counts of pulmonary endocrine cells were always evidenced just prior the birth, then their counts declined during the neonatal period, and only rare endocrine cells were observed in the adult stage [9, 11].

Serotonin is the principal amine produced by PNEC system. The appreciable amount of serotonin in PNEC system of rabbit and rat were found mainly in foetal and neonatal lung [9, 28, 40]. In the present study, serotonin positive cells were also mainly observed in late foetal and early neonatal lungs, and thereafter the numbers of these cells were shortly decreased for reaching minimal frequency during the adult period. Based on these observations, serotonin may be an important mediator in the foetal and early neonatal rat lung, involved in the process of lung development or in the regulation of the smooth muscle tone in airways and pulmonary vessels either in utero and possibly during adaptation to air breathing at birth [11, 33].

CGRP is a 37 amino-acid peptide coded by the calcitonin gene. In the respiratory tract, the presence of CGRP immunoreactivity was reported in guinea pig, human and mouse [8, 39] and was localised either in nerve fibers or in neuroendocrine cells. The precise function of CGRP in the lung is largely speculative. Its appearance during foetal life [7, 21] and the fact that there is a higher density of CGRP containing endocrine cells in the lungs of foetal and newborn animals than in the lungs of older ones reported in the present study and by other researchers [26] suggest that CGRP structures may be functional in the foetus and may be involved in the pulmonary adaptation at birth for rapid vasodilatation upon aeration of the lung. In addition to localization of CGRP immunoreactivity, the present study was also demonstrated calcitonin immunoreactivity in PNEC system of developing rat lung. But our result indicated that endocrine cells containing calcitonin were different from the CGRP positive cells because of their different distribution and because calcitonin immunoreactivity was largely unaffected during the lung development. Similar results were also obtained in developing rat lungs in previous studies [25].
CCK immunoreactivity has been reported in pulmonary neuroendocrine cells and neuroepithelial bodies of monkey, dog, sheep, rat and hamster [41]. In these species, the distribution and intensity of CCK positive cells varied. In agreement with a previous study [5], CCK immunostaining in the present study appeared less intense in the rat lung.

Somatostatin is most known for its localization to pancreatic islet D cells [4], but it has also been detected in pulmonary neuroendocrine cells and nerves of foetal rhesus monkey [14]. In the present study, low somatostatin immunoreactivity was found in pulmonary endocrine cells whatever the lung development period and our findings are in agreement with the rare investigations previously made [3, 5, 6, 31].

In the present study, VIP immunoreactivity was not found in pulmonary endocrine cells during the development of the rat lung. VIP immunoreactivity has been reported to be only present in nerve fibers along smooth muscle, blood vessels and glandular tissue of the lung in cat, rat and human [15, 16, 19, 23, 27].

In conclusion, the present study has revealed that serotonin and CGRP may be prominent mediators in foetal/neonatal lungs and their role may be particularly important during lung development and neonatal adaptation.

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References


Table I. — Relative densities of serotonin and peptide-containing endocrine cells in rat lung during ontogeny.

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