to them, with MAO inhibitor pretreatment DA caused a small increase in the number of fluorescent cells also in these parts of the pituitary gland. Our study of the neural lobe after dopamine injections combined with various drug pretreatments is in progress. It seems that the uptake of dopamine in the neural lobe is specific for the cells and processes originating from the basal hypothalamus.

The decrease of the intensity of the FIF with the age of the rat coincides with simultaneous increase in the MAO activity. However, the decrease in the uptake of dopamine with age may represent a change in the membrane permeability: many embryonal cells are capable of taking up substances which they cannot take up at an adult age. In the neural lobe, there are no ordinary synapses between monoamine-nerves and neurosecretory axons and pituicytes[4]. It is possible that dopamine can be taken up by the neurosecretory axons and pituicyte membranes. This would explain the universally distributed fluorescence after dopamine injection.

Zusammenfassung. Die Aufnahme von injiziertem Dopamin wurde mit Hilfe der Fluorescenzmikroskopie in der neurosecretorischen Axone und in den Pituizyten des Hypophysenhinterlappens der Ratte nachgewiesen. Die Intensität der Fluorescenz nahm während der postnatalischen Entwicklung ab. Mögliche Erklärungen über diese Erscheinung wurden diskutiert.

S. PARTANEN and LEENA RECHARDT

Department of Anatomy, University of Helsinki, Siltavuorenpenger 20, SF-00170 Helsinki 17 (Finland), 26 March 1973.

Intrapulmonary Neuro-Epithelial Bodies: Hypoxia-Sensitive Neuro(Chemo-)Receptors

The possibility that intrapulmonary air chemoreceptors, in addition to the well established central and peripheral chemoreceptors, play a role in the regulation of the lungs has been a fundamental but unanswered problem for the past 20 years[1-3]. They had not been identified histologically by 1971[4], even though physiologic evidence of their presence was available[5]. It was indeed well established that hypoxia causes a pulmonary vasoconstriction with the aid of an intrapulmonary receptor[6]. A major influence of the central nervous system (CNS), the arterial pH and lactic acid on this system was excluded[7], while serotonin could be mediating it[8].

We have recently identified[9,10], the locus, architecture and basic structure of so-called neuro-epithelial bodies (NEBs) within the bronchial, bronchiolar and even alveolar epithelial lining cells of the mammalian lung (including man). They are located near fenestrated bronchial capillaries. We postulated them to be intrapulmonary chemo-, stretch-, baro- or tactile neuroreceptor organs modulated by the central nervous system which exhibit local secretory activities. We proved indeed by electron microscopic cytochemistry and microspectrography that these corpuscles contained serotonin amongst other substances[10].

In this report we demonstrate that the neuro-epithelial bodies of the intrapulmonary lining epithelium of rabbits secrete their dense-cored, serotonin-containing vesicles (DCV's) at their basal vascular pole after exposure to hypoxia. We propose that amongst their various possible neuroreceptor functions the NEBs provide an intrapulmonary, hypoxia-sensitive chemoreceptor system in addition to the well-established central and peripheral (e.g. carotid body) chemoreceptors. They secrete serotonin

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Number	O_2 (%)	Duration (min)
2	5	2
2	5	10
2	5	20
2	10	2
2	10	10
2	10	20
2	15	2
2	15	10
2	15	20

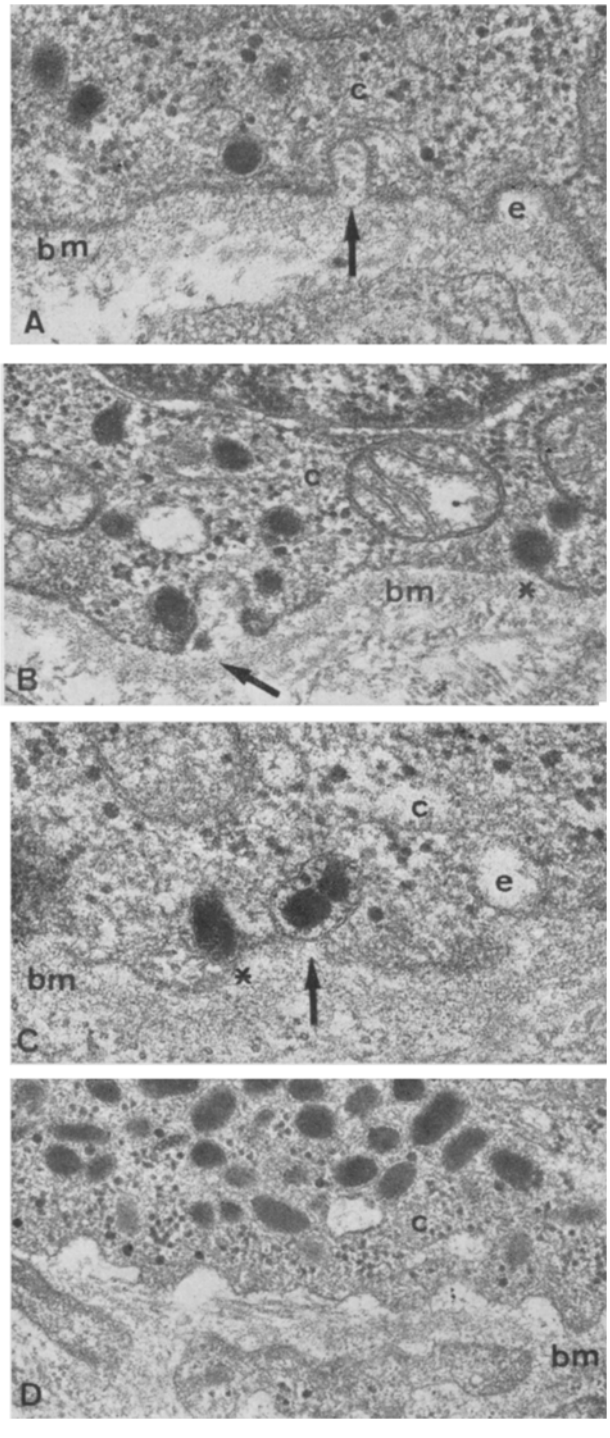
Number of neonatal rabbits submitted to various degrees of hypoxia during different periods of time.

→

Fig. 1. All figures illustrate various stages of the exocytosis cycle of DCVs of the granulated cells of the NEBs under hypoxic conditions; they illustrate in each substance a part of the basal area of the granulated cell cytoplasm (c), their basement membrane (bm) and the immediately adjoining subepithelial extracellular space. A) Exocytosis (arrow) of a DCV which still contains small fragments of its dense core; e, empty DCV; other DCVs are in the basal cell cytoplasm; neonatal rabbit; glut. fix. with postosmification; uranyl acetate and lead citrate staining; 10 min hypoxia with 10% O_2. ×64,638. B) DCV (x) contacting and fusing with the basal cell membrane; exocytosis of a DCV still containing some fragments of its dense core (arrow); same methods as in 1.A); ×48,988. C) Before extruding their contents DCVs, sometimes fuse (arrow); DCV (x) making contact with the basal cell membrane; e, empty DCV; same methods as in 1. A). ×76,608. D) Remarkably undulating basal cell membrane due to the exocytosis in the extracellular space of several DCVs, which are already empty; this is seen after a short hypoxia; same fixation and staining methods as in 1. A); 2 min hypoxia with 10% O_2. ×47,082.

and probably also related amines or peptides which influence the pulmonary vasoconstrictor response and are modulated by the CNS.

Material and methods. We took lung biopsies of 18 neonatal term rabbits which were decapitated within an airlocked cage in which they had stayed, 2 by 2, for a variable time interval under various concentrations of oxygen, as indicated in the Table. The oxygen concentration was continuously monitored and kept constant with a Beckman Oxygen Analyser 7.700 through controlled inlets of oxygen and nitrogen. 2 adult rabbits and 6 neonatal term rabbits served as control.



For light microscopy the tissues were fixed in Bouin's fluid, embedded in paraffin, serially sectioned and stained with the usual techniques. Argyrophilia was detected according to Bodian's silver proteinate technique as modified by VAN CAMPENHOUT, and GRIMELIUS' silver nitrate technique[11] and argentaffinity according to the Fontana-Masson technique. We also investigated tissues with FALCK's histochemical fluorescent amine technique as in our earlier studies[9,12]. For electron microscopy, biopsies were immediately fixed in 2.5% glutaraldehyde (0.1 *M* phosphate buffer, pH 7.2), followed by osmification. Sections (1 μ) cut from the Epon embedded blocks were stained with toluidine blue. As the NEBs are distinctly visible on such sections, the blocks were trimmed and correlated for electron microscopical studies (Philips EM 300) carried out on the immediately adjacent ultrathin sections, stained with uranyl acetate and lead citrate[13].

Results. Using these various light optical, histochemical and electron microscopical techniques, we observed in all animals[9,10] the widespread occurrence within the intrapulmonary lining epithelium of argyrophil, argentaffin, yellow fluorescent, ultrastructurally granulated and innervated epithelial cellular organs. No basic morphologic difference exists between the NEBs of neonatal and adult rabbits.

In all animals exposed to hypoxia, electron m croscopy revealed a distinct and pronounced exocytosis of the corpuscular DCVs at the level of the adepithelial basement membrane; this phenomenon is only rarely observed in the control and normal[10] animals. All classic morpholo-

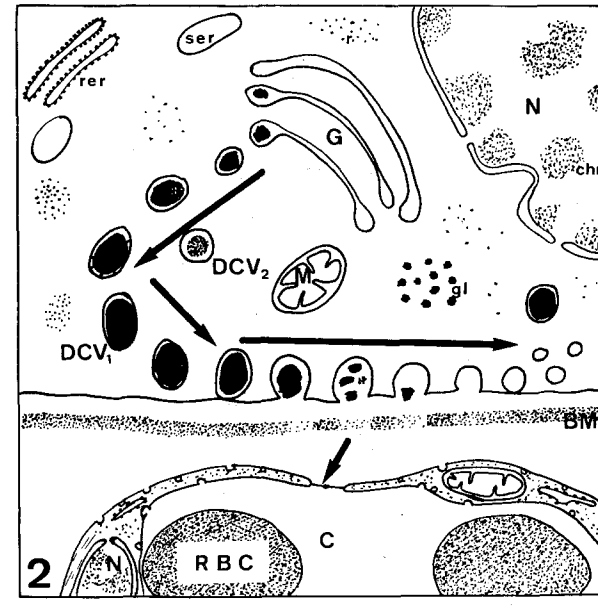


Fig. 2. Scheme illustrating the different stages observed during the exocytosis cycle of the DCVs of the granulated cells of NEBs submitted to hypoxia. DCV_1, dense cored vesicle of the first type; DCV_2, dense cored vesicle of the second type. BM, basement membrane; C, capillary; chr, chromatin; G, golgi system; gl, glycogen granules; M, mitochondria; N, nucleus; RBC, red blood cells; rer, rough endoplasmic reticulum; ser, smooth endoplasmic reticulum.

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gical phases of the exocytosis cycle[14] are seen (Figures 1 and 2); besides the usual and filled or granulated DCVs dispersed throughout the cytoplasm (preferentially the subnuclear areas) of the epithelial cells of the NEBs, numerous DCVs concentrate in a much larger number than in the control animals in the vicinity of the basal cell membrane; next DCVs are observed whose membranes become fused with the cell membrane itself (Figure 1, B,C). Consecutively they open at the level of the basal membrane with an extrusion and exocytosis of their contents into the space between the basal cell membrane itself and the basement membranes. At this stage of the secretory cell process, the vesicle may still be observed to contain a small dense core which may become fragmented (Figure 1, A,B), or the vesicle may appear entirely empty (Figure 1, A,D). Finally, we observed areas of the basal epithelial cell cytoplasm which contained vesicles both empty and smaller than the classic DCVs and which are not seen in the normal state. They probably correspond to so-called 'refilling' vesicles[14]. Besides the exocytosis, hypoxia treated animals reveal occasionally a slight and focal mitochondrial lysis and a pronounced development of the Golgicomplex, which is located above the cell nucleus and forms small DCVs and many empty small cisternae.

Discussion. As the apical cell pole of the NEBs immediately contacts the airway lumen and its contents on the one hand, and as a fenestrated blood capillary is closely apposed to their basal or vascular pole on the other hand[10], it appears logical that the NEBs are chemoreceptor organs with a local intrapulmonary secretory activity, one of the substances released within the blood stream of the lungs being serotonin. This identifies the previously unelucidated, intrinsic morphological mechanism explaining the occurrence of a hypoxia induced pulmonary vasoconstriction[1–3] which is in itself not markedly influenced by the nervous system, blood pH and lactic acid, but mediated by humoral substances[6,7], e.g. serotoin[8]. NIDEN et al.[15] have demonstrated that serotonin injected into the pulmonary circulation causes an increase in the oxygen saturation of the pulmonary venous blood. As most of the intrapulmonary bronchial capillary and venous blood is drained off via the pulmonary circulation[16], it may well be that the serotonin secreted by the NEBs during hypoxia causes a vasoconstrictor response with blood shunting from the poor to the better oxygenated and ventilated portions of the lung, providing besides the central and peripheral chemoreceptors a third or locally inbuild intrapulmonary chemoreceptor system which finely adjusts the ventilation to perfusion ($\mathring{V}/\mathring{Q}$) ratios.

Résumé. Les «Corpuscules Neuro-épithéliaux» de l'épithélium respiratoire intrapulmonaire ont ont été étudiés au microscope optique et électronique chez des lapins soumis à des conditions expérimentales d'hypoxie. Dans ce cas ils sécretent à leur pôle vasculaire basal leurs vésicules à noyau dense contenant de la sérotonine. Nous supposons que parmi leurs diverses fonctions neuroréceptrices possibles, ces «Corpuscules Neuro-épithéliaux» forment un système intrapulmonaire chémorécepteur sensible à l'hypoxie, en plus des chémorécepteurs centraux et périphériques (par exemple le corps carotidien) dont l'existence est bien connue. Ils sécretent de la sérotonine et probablement aussi des substances aminées ou peptidiques associées qui influenceraient la vasoconstriction pulmonaire et sont modulés par le système nerveux central.

J. M. LAUWERYNS and M. COKELAERE[17]

Katholieke Universiteit te Leuven, School of Medicine, Laboratory of Histopathology, 12, Minderbroedersstraat, B–3000 Leuven (Belgium), 13 August 1973.

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Electron Microscopy of the Effects of Histamine and Thermal Injury on the Blood and Lymphatic Endothelium, and the Mesothelium of the Mouse's Diaphragm, Together with the Influence of Coumarin and Rutin[1]

MAJNO et al.[2] showed that 3 mediators of inflammation (including histamine) cause contraction of the endothelial cells of venules. Subsequently[3] a combined light and electron microscopic approach has shown that the contracted cells are those associated with the open junctions which cause the increased permeability of these vessels. In thermal injury, COTRAN[4] showed that the affected cells and open junctions occur in capillaries rather than venules. This has been ascribed to the direct affects of the injury[5,6]. We therefore decided to study the effects of histamine and thermal injury on the contraction of the endothelial cells in both classes of vessels in the diaphragm of the mouse.

Any form of injury which has been tested has been shown to result in the opening of many junctions in the endothelium of the initial lymphatics[7]. There is much evidence indicating that this is largely because of the effects of oedema pulling these vessels open and their cells apart, together with other factors occurring in normal lymphatics[7]. However, because lymphatic endothelium is so similar to that of blood vessels, we decided to examine the effects of the injuries on lymphatic endothelial contraction. Similarly, because of the similarity of the mesothelium to endothelium[8], we decided to examine this too, using that adjacent to the injurious stimuli.

It has recently been shown that coumarin and related compounds have the property of considerably reducing high-protein oedemas, including those caused by thermal injuries[9–14]; it is considered that this probably occurs because coumarin induces considerable proteolysis of the extravasated plasma proteins in the tissues[11,12]. While it