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小鼠肺內支氣管肌肉之電子顯微鏡研究

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摘　要

本研究是以穿透式電子顯微鏡來觀察小鼠肺內支氣管平滑肌肉細胞之細微構造，小鼠肺內支氣管肌肉細胞之細微構造與其他動物氣管或其他組織的平滑肌肉細胞相類似。平滑肌肉細胞成環狀排列圍繞於支氣管外側，細胞形狀為長條梭形，彼此之間依長軸平行排列，細胞核為長橢圓形，位於細胞中央最寬大處，經過細胞核之細胞橫切面的直徑約 4.6μm，其他胞器如線粒體、內質網膜、高氏體及微管等都可觀察到。關於平滑肌肉細胞之收縮纖維目前只見到直徑約 5nm 之肌動蛋白絲，及直徑約 10nm 之中間型纖維，並未發現最粗的肌凝蛋白絲，緻密體、緻密帶及肌肉膜泡的構造皆與其他平滑肌肉細胞相似。在電子顯微鏡下可分辨出三種細胞連合情形：間隙連合構造、中間型連合構造及手指狀突起。

前　言

呼吸道平滑肌肉與某些呼吸性疾病有關，患有氣喘的病人其呼吸道平滑肌肉細胞有增生及肥大的現象[1]，而慢性支氣管炎也有類似的情形產生[2]，其原因不明。呼吸道肌肉體積增加，理論上會引起呼吸道窄化，而呼吸道肌肉的收縮，會引起支氣管痙攣管徑縮小。最近有一些假說顯示氣喘時平滑肌肉本身功能正常，而是控制肌肉的神經，或是抗原抗體反應所釋放出來的化學物質異常，而使得平滑肌肉過度收縮所導致[3,4,5]，但目前並無適當的動物模式可以來研究呼吸道平滑肌肉在病態時的構造及功能[6]。

平滑肌肉的功能與其構造有關，許多有關動物氣管平滑肌肉細胞細微構造的研究報告已經發表，包括大鼠[7,8]、犬[9,10]、牛[11]、人[12]等，一般氣喘時，氣管和極細小的支氣管不會收縮，只有中間型支氣管會有收縮的現象，但對於支氣管平滑肌肉細胞構造的研究卻不多見，本實驗是利用穿透式電子顯微鏡來觀察小鼠肺內支氣管平滑肌肉細胞的細微構造，以與其他動物作比數。

材料與方法

選用 10 隻 ICR 品系體重 26-28g 之健康小白鼠，雌雄各半，將每隻小白鼠以斷頸法殺死，剪開胸腔及橫隔膜，使氣管露出，以 1 毫升之針筒配以 1 英寸長 25G 之針頭吸取固定液〔固定液為 0.1M 二甲次砷酸鹽緩衝液(sodium cacodylate buffer)配製之 3% 戊二醛(glutaraldehyde)溶液 pH7.4, 4℃〕，由喉下氣管注入至肺部脹起貼到肋骨時為止，注射量約 0.4-1.0ml，這時用將氣管上端用棉線紮緊，然後在解剖顯微鏡下剪取整付肺臟組織，置於相同新鮮的固定液中固定 1 小時後，再將左肺剪下取肺門部位之組織切成許多 $1mm^3$ 的組織塊，浸入裝有新鮮固定液的小瓶中另固定 1 小時是為前固定，之後用 0.1M 二甲次砷酸鹽緩衝液將組織小塊涮洗三次，各 15 分鐘，再將組織小塊置入 2% 鐵酸(sodiun tetroxide)中固定 1 小時，所有的固定作用都是在 4℃ 的溫度下進行。當組織固定後則用一系列不同濃度的酒精脫水，最後把組織放入包埋用膠囊(Micron Product, Canada)，內倒入環氧樹脂(Epon 812)加以包埋，將包埋膠囊置入 60℃ 的溫箱裏放置 2-3 日，當環氧樹脂聚合成組織塊後即可取出準備切片。

將樹脂組織塊用刀片修整後，先以超薄切片機 (Ultracut E, Reichert-Jung, Austria) 切成 0.5um 厚的厚片，以甲苯俊藍(toluidine blue)染色，在光學顯微鏡下觀察厚片等找到有肺內支氣管後，再將組織塊作進一步修整，去除多餘無用的組

織，最後用鑽石刀將組織作超薄切片，厚度約 70-90nm，以 200 或 300mesh 之銅網片將超薄切片撈起，置放於濾紙上陰乾，然後以醋酸鈾(uranyl acetate)及檸檬酸鉛(lead citrate)(Reynolds 1963)進行雙重染色，將銅網片放入電子顯微鏡(Hitachi H-600, Japan)觀察支氣管平滑肌肉的細微構造，把所觀察到適當的支氣管平滑肌細胞以不同的倍數拍攝成顯微底片，最後將所有底片以 2.7 倍放大成電子顯微照片以供研判、測量及比對之用。

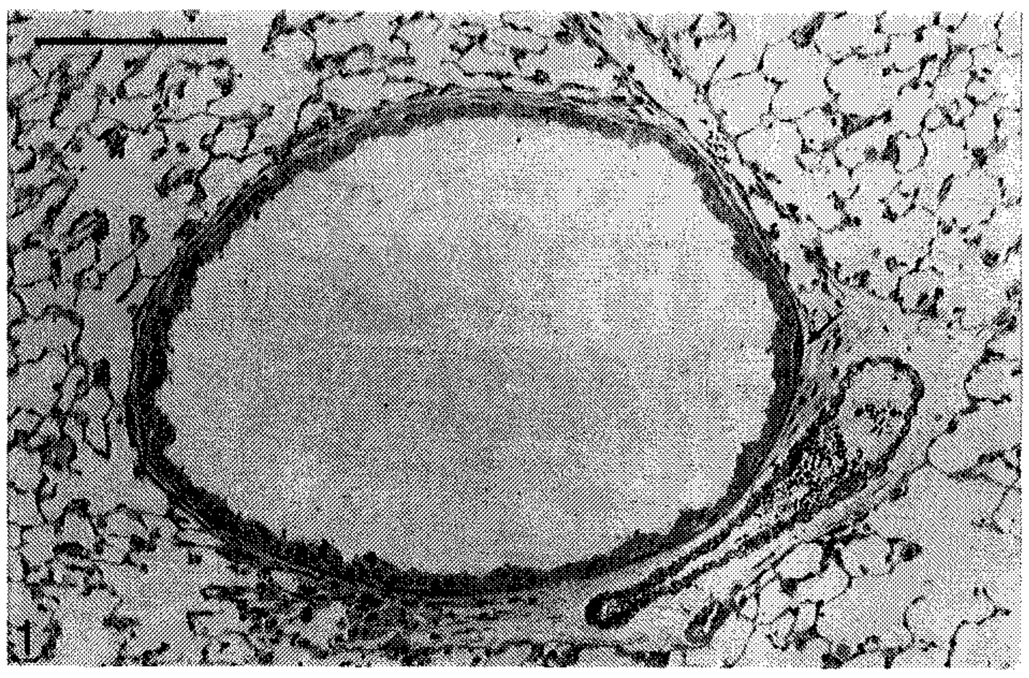

Figure 1. The transverse section of the mouse intrapulmonary bronchus. The epithelium is the simple cuboidal epithelium. 1-3 layers of smooth muscle cells surround the bronchus. The nuclei of two smooth muscle cells are visible (arrow). The section was stained by toluidine blue. Calibration bar = 0.1mm.

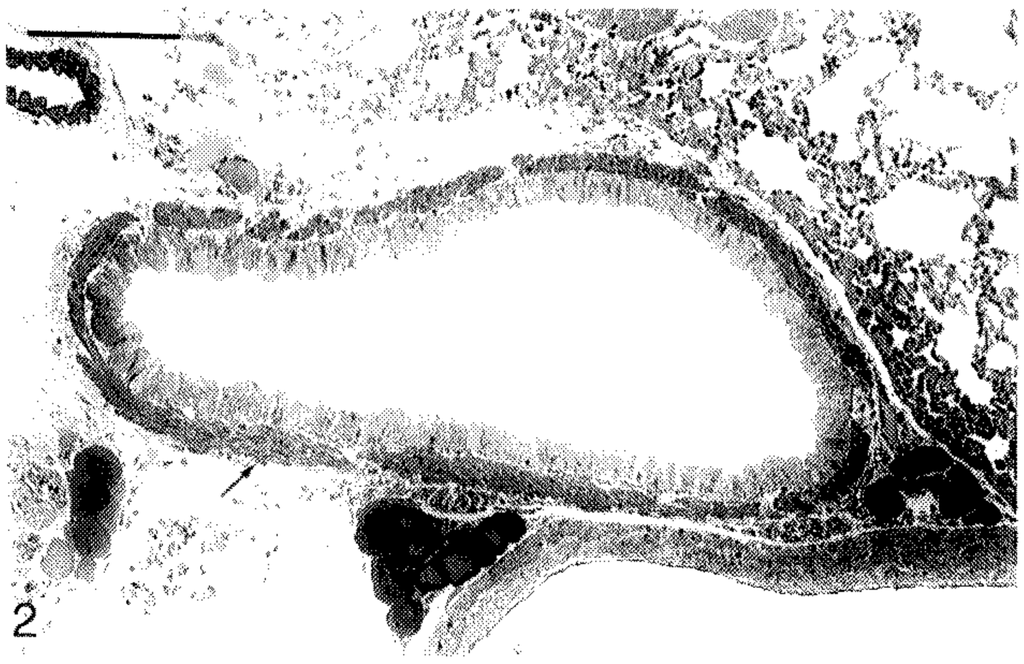

Figure 2. The longitudinal section of the mouse intrapulmonary bronchus. The epithelium is a ciliated low columnar epithelium. 5-8 layers of smooth muscle cells surround the bronchus(arrow). on the lower right corner of the photomicrograph, there is a pulmonary vein. The section was stained by toluidine boue. Cali-bration bar = 0.1mm.

結　果

以光學顯微鏡觀察小鼠肺臟組織樹脂厚片，可發現小鼠肺內支氣管的上皮由有絨毛矮柱狀上皮逐漸轉變為簡單立方形上皮，支氣管周圍有許多平滑肌肉細胞圍繞成環狀排列，接近肺門部位的支氣管內徑約 0.5mm，周圍肌肉層約有 10 層肌肉細胞重疊排列(圖二)，當管徑逐漸減小，肌肉層也隨之變薄，當管徑約為 0.4mm 時，可發現有 4 至 6 層肌肉細胞圍繞，管徑為 0.3mm 時，細胞層則減少為 1 至 3 層肌肉細胞(圖一)，支氣管內徑小至 0.1mm 時，就發現不到任何平滑肌肉細胞的存在。支氣管平滑肌肉細胞的形狀呈細長條兩端尖細的梭形，細胞核位於肌肉細胞中央最寬處，

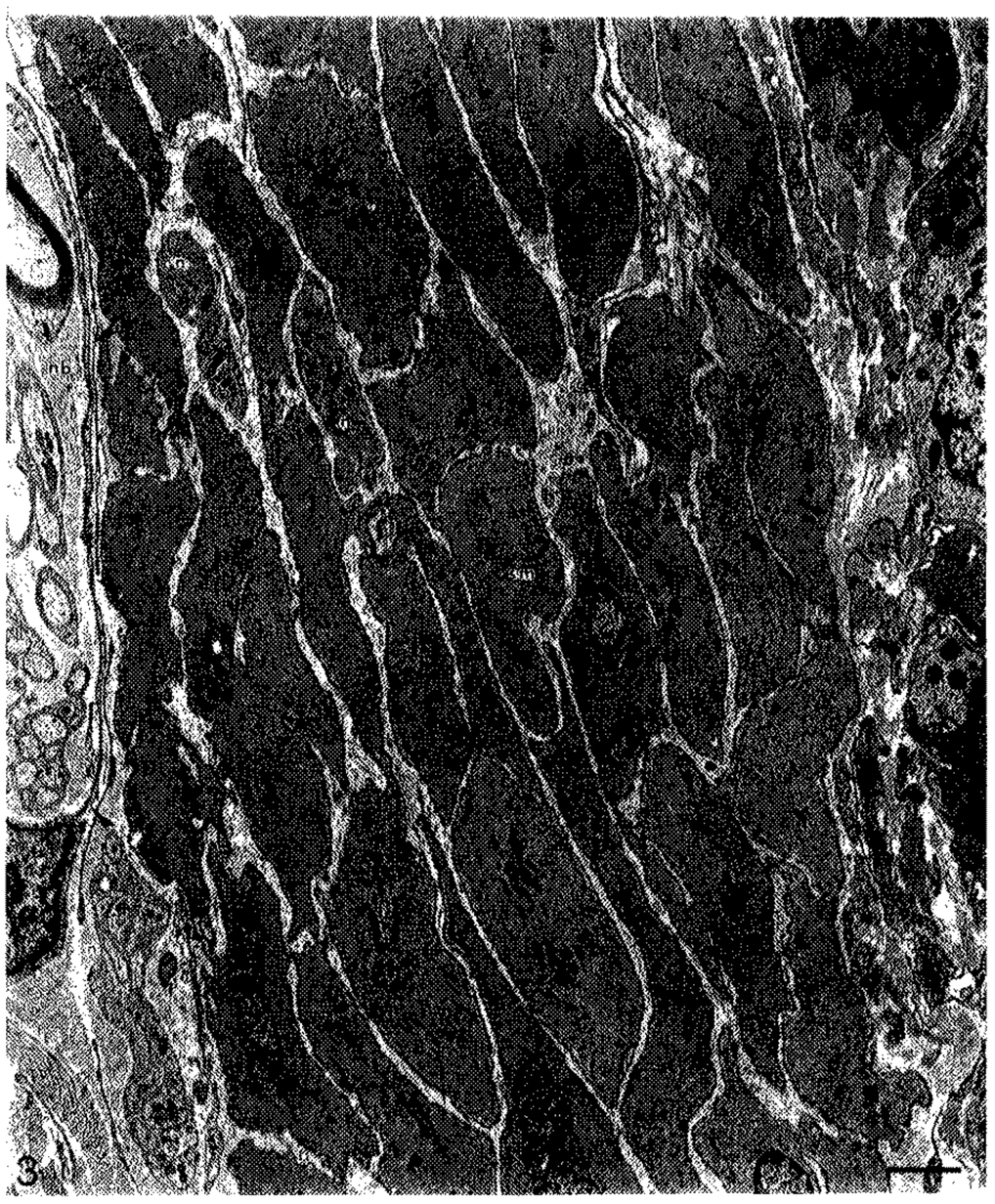

Figure 3. The transverse section of the mouse intrapulmonary bronchus. Many smooth muscle cells arrange parallel to each other at the middle of the photomicrgraph. The right side is the epithelial cells (epi)of the bronchus. on the left side, a big nerve bundle (nb) and a nerve fiber (n) distribute to the outer surface of muscle layer. Two nerve fibers (n) are present berween smooth muscle cells. The big nerve bundle compose of many myelin-ated and amyelinated nerve fibers. Nerve fibers are encapsulated by the process of fibroblast (arrow). A process of interstitial cell (ic) penetrates between smooth muscle cells. Calibration bar = 2μm.

Figure 4. The oblique section of smooth muscle cells of the mouse intrapulmonary bronchus. Muscle cells are spindle in shape and arrange parallel to each other. The nucleus is cylinder in shape and locate in the middle of the cell. A gap junction (arrow) is visible between two muscle cells. A nerve fibre (n) is present. Calibration bar = 1μm.

Figure 5. The transverse section of smooth muscle cells of the mouse intrapulmonary bronchus. several mitochondria (mit) locate in the middle of muscle cell. Many dense bodies (db) scatter throughout the cytoplasm. Microtubules (mt) are also visible. The presence of dense bands can be seen on the cell membrane. Cavaeo-lae (arrowhead) are arranged between dense bands. Smooth muscle cells are covered by a layer of basal lamina. Many collagen fibrils are present at the left side of smooth muscle cells. Calibration bar = μm.

在光學顯微鏡下可以明顯地分辨出(圖二)。

用電子顯微鏡可觀察到支氣管壁上肌肉層內平滑肌肉細胞之間呈長軸平行排列(圖三),肌肉層外側有時可見大型有髓鞘或無髓鞘神經束分布,小神經纖維大多分布於支氣管肌肉層外側,少數分布於支氣管上皮與肌肉層之間,在管徑較大的支氣管肌肉層細胞中間,也可發現一些神經維的存在,同時有間質細胞突起分布(圖三)。支氣管平滑肌肉細胞的細胞核為長橢圓形,核中央大部分是正核染質(euchromatin),而異核染質(heterochromatin)則成小塊狀散佈於細胞核四周的核膜下(圖四),有時可見到一個明顯的核仁,一個平滑肌肉細胞只含有一個細胞核,具有兩個細胞核的情況極為罕見(圖七)。平滑肌肉細胞的橫切面大致為橢圓形,在細胞最寬處含細胞核之切面直徑約 4.6μm。

支氣管平滑肌肉細胞的細微構造與其他器官的平滑肌肉細胞相似,細胞質內有橢圓形的線粒體位於細胞核的兩端或聚集在細胞中央(圖五),直徑約 0.2μm,內質網膜及高氏體等胞器較為少見,細胞質各處都可見到微管(microtubule),直徑約 20nm,在平滑肌肉的橫切面上可以觀察到兩種粗細不同的肌纖維,一種為直徑約 5nm 的細纖維,稱為肌動蛋白絲(actin filament),另一種為直徑為 10nm 的中間型纖維(intermediate filament),數量較少,至於一種最粗的肌纖維肌凝蛋白絲(myosin filament)則未發現。散佈於細胞質中有許多一團團電子濃密聚集的構造,大小不一,平均直徑約 0.6μm 稱為密集體(dense body),而在細胞膜內側的某些部位也有類似的帶狀構造,長度不等,厚度約為 0.3μm,稱為密集帶(dense band),這兩種構造似乎都有肌動蛋白絲穿入。介於密集帶之間的細胞膜上有一些成串排列整齊的小泡構造,大小相似,形狀類似三角錐瓶,深度約 100nm,寬度約 80nm,頸部只有約 50nm 寬(圖五),稱為肌肉膜泡(caveolae intracellularis),當切面切至肌肉細胞表面時,可以見到這種小泡狀的構造整齊排列成行(圖六)。

支氣管平滑肌肉細胞外表被一層絨毛樣的基底膜所覆蓋,厚度約 30nm,細胞外有許多直徑約 10nm 的微絲(microfilament)與基底膜相連(圖六),每個細胞周圍都有結締組織膠原纖維(collagen fibril)分布(圖五,圖七),其直徑約 30-50nm,這種纖維的走向大致與平滑肌肉細胞的長軸平行。

在電子顯微鏡下可觀察到有些平滑肌肉細胞之間藉一種間隙連合構造(gap junction)相連,其細微構造為兩層細胞膜之間有一個 2-3nm 的間隙相隔(圖八,圖九),有的肌肉細胞之間相隔約

Figure 6. The tangential section of the end portion of a smooth muscle cell. Many cavaeolae are arranged regularly (arrowhead). Many microfilaments connect to the cell membrane of a smooth muscle cell. Calibration bar = 1μm.

Figure 7. Two nuclei are present in one smooth cell of the mouse intrapulmoary bronchus. Many mitochondria (arrow) surround one nucleus. bar = 1μm.

70nm, 相對稱的細胞膜上都有緻密帶的構造, 細胞膜間的兩層基底膜愈合為一層(圖十), 這種構造稱為中間型連合構造(intermediate junction), 有時可見到一個平滑肌肉細胞會伸出像手指樣的突起與另一個細胞的突起互相勾連在一起(圖八)。

小鼠肺內分布於支氣管及肺靜脈上的微血管均為無窗型微血管(圖十一, 圖十二), 內皮細胞間藉緊密連合(tight junction)相結合。

討　論

小鼠肺內支氣管平滑肌肉細胞的細微構造與其他動物的氣管肌肉細胞及其他組織的平滑肌肉細胞非常相似, 支氣管平滑肌肉細胞圍繞於支氣管外側, 彼此之間依長軸成平行排列, 細胞層數依管徑大小而遞減, 而氣管平滑肌肉細胞只存在於氣管的背側, 細胞層數不變, 細胞平行排列與支氣管肌肉細胞相同。

在本實驗中小鼠肺內支氣管平滑肌肉細胞的長度不易測量, 由他人的研究得知, 牛的氣管肌肉細胞長度約 806μm[11], 犬的氣管肌肉長度約 1000μm[10], 一般動物腸子的平滑肌肉細胞長約

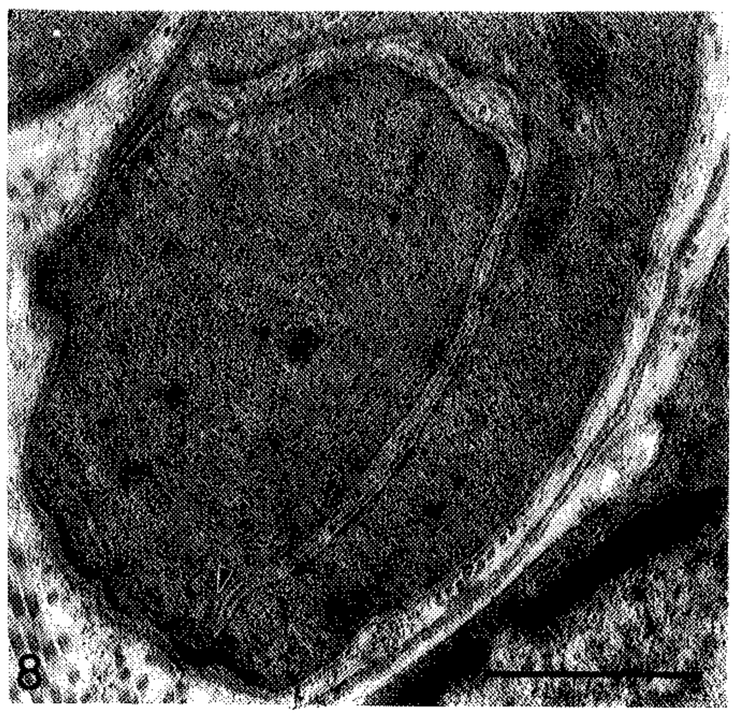

Figure 8. One end of a smooth muscle cell attach with another muscle cell with a gap junction (arrow), and the other end forms a small process closely cross with a small process of the same other muscle cell. Calibration bar = 1μm.

Figure 9. Two smooth muscle cells touch together with a gap junc-tion (arrow). Calibration bar = 0.5μm.

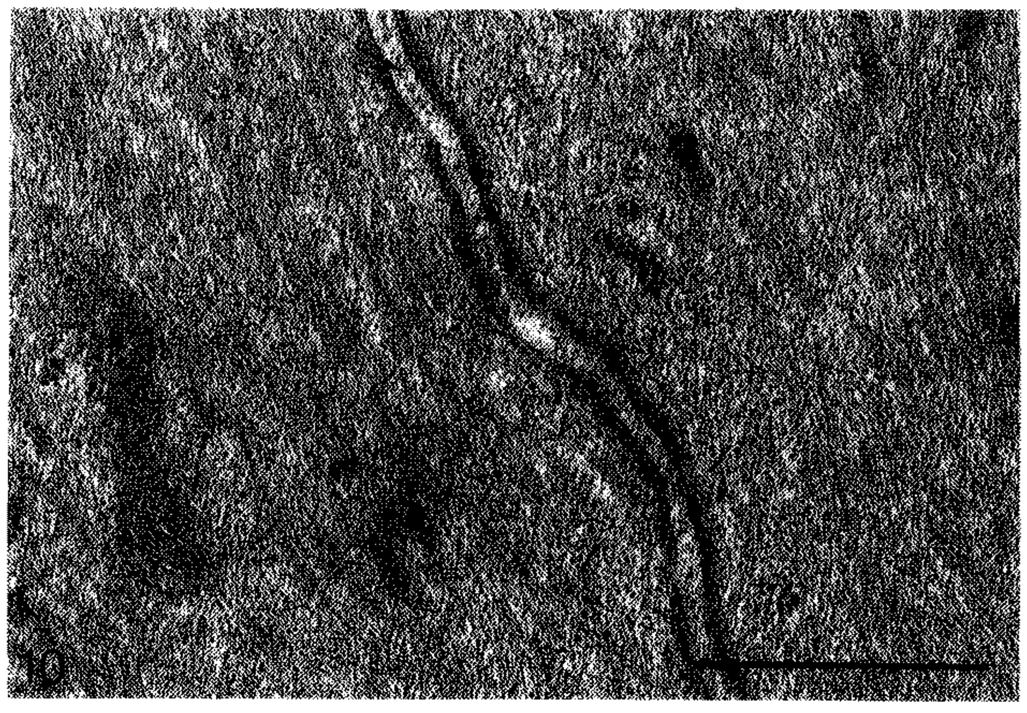

Figure 10. Two smooth muscle cells appose closely with an intercel- lular gap of approximately 30 nm. The membranes are occupied by electron dense material. Two layers of basal lamina fuse together to form a single ones. Calibration bar = 1μm.

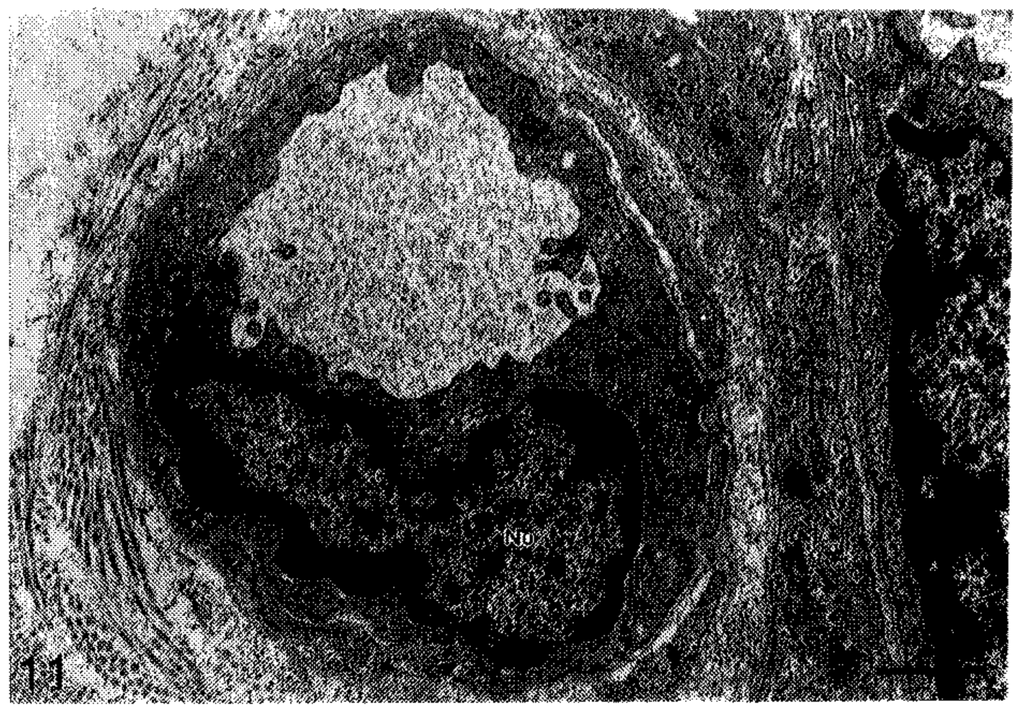

Figure 11. The transverse section of a capillary of the mouse intrapulmonary bronchus. The nucleus (Nu) of the endothelial cell is visible. This is a continuous type of capillary. Calibration bar = 1μm.

400-600μm 之間[13], 因此不同平滑肌肉細胞的長度差異很大。小鼠肺內支氣管平滑肌肉細胞切過細胞核的切面其細胞直徑約 4.6μm, 這與牛的氣管肌肉細胞相似(4-8μm)[11] 。

小鼠肺內支氣管平滑肌肉細胞質內所含的線粒體直徑約 0.2μm, 與其他平滑肌肉細胞相似[14], 牛氣管平滑肌肉細胞內線粒體約佔細胞體積之 2.4%, 比其他組織平滑肌肉細胞的數值為低[14,15] , 一般認為線粒體在平滑肌肉中有吸收及聚集鈣離子的作用[16], 在支氣管平滑肌肉細胞質內可見到許多微管散佈其間, 至於其功能則未知。在本實驗中只觀察到肌動蛋白絲及中間型絲分布於支氣管平滑肌肉細胞質當中, 而未發現肌凝蛋白絲的存在, 可能是由於組織在製備過程中失去這種蛋白質, 這種現象相當普遍[5], 然而處理良好的平滑肌肉細胞依舊可見到肌凝蛋白絲, 直徑約 15nm[13], 中間型肌絲直徑約 10nm, 大小介於肌動蛋白絲與肌凝蛋白絲之間而得名, Cooke 認為這種肌絲在平滑肌肉細胞內形成一個支持網路, 有細胞骨骼(cytoskeleton)的作用[17]。在電子顯微鏡下可以見到肌動蛋白絲穿入緻密體及緻密帶, 兩者關係密切, 這種現象在多年前即有報告[18], 有人認為緻密體與肌動蛋白絲的結構關係類似骨骼肌的 Z 線, 與平滑肌肉的收縮有關[19]。支氣管平滑肌肉細胞周圍有許多膠原纖維, 同時在細胞末端發現有許多微絲與細胞膜上的緻密帶相連, 由形態學的觀察, 某些部位細胞末端發現有許多微絲與細胞膜上的緻密帶相連, 由形態學的觀察, 某些部位的細胞膜內與肌動蛋白絲相連, 外與微絲及膠原纖維相接, 這可能構成一種力量傳動的組合, 因此肌肉細胞的收縮會影響到周圍的組織。

肌肉膜泡在其他動物的氣管平滑肌肉細胞膜上都可發現[5,10,11], 而且為不同組織所有平滑肌肉共同具有的特徵, 細胞膜泡與細胞外界相通, 並不具有胞飲的作用[20], 至於其真正功能尚未完全了解。

在本實驗中可以觀察到支氣管平滑肌肉細胞之間有三種連合方法, 其中間隙連合構造在動物的氣管及腸子平滑肌肉細胞間都可見到, 相連兩個細胞當中的離子及小分子都可以藉這個構造互

Figure 12. A continuous capillary is found near the pulmonary vein. The opposed cell mimbranes of two endothelial cells fuse to form a tight junction (qrrow). Mitochondria (mit) are arranged under the sarcolemma of pulmonary vein with the fine structure similar to cardiac muscle. Calibration bar = 1μm.

相交流,因此形成離子或新陳代謝性聯結,許多研究人員認為間隙連合構造為肌肉細胞之間電聯結的部位,在一般電子顯微鏡照片上間隙連合構造顯示兩層細胞膜間的距離約 2-3nm, 呈透明狀,其實兩層膜之間有一些平行排列的物質與膜結合[13]。人的氣管每 100 個平滑肌肉細胞有 2.7 個間隙連合構造,支氣管有 2.2 個[12],牛有 8 個[11],而犬[10]及天竺鼠[6,8]則很少見,小鼠、大鼠、兔子及羊的氣管平滑平滑肌肉細胞間隙連合構造的數目都不相同(Chiang and Gabella, unpublished data),因此這種構造的數量在不同動物之間有很大的差異,其數目似乎與細胞間是否具有電聯結無關,支氣管平滑肌肉細胞手指狀的突起不但用穿透式電子顯微鏡可以觀察到,最近有人利用掃描式電子顯微鏡也證實腸子平滑肌肉細胞也有類似的突起,會穿入另一個細胞裏[21]。

嚙齒類動物肺靜脈的管壁由心臟肌肉構成早已有人報告[22,23],本實驗只是再次證實小鼠之肺靜脈其細微構造確屬心肌結構。

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The Intrapulmonary Airway Smooth Muscle of Mice an Ultrastructural Study

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ABSTRACT

The fine structure of intrapulmonary airway smooth muscle of mice was studied by transmission electron microscopy. The fine structure of bronchial smooth muscle cells is similar to that of trachea of other mammals and similar to that of other organs. The smooth muscle cells surround the bronchus like a ring. They are arranged parallel to each other along their long axis. The nueleus is oval in shape and locate in the widest part of the cell. The diameter of the cell profile cutting through the nucleus was about 4.6 um. Other organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus and microtubules were also examined. Only one type of contractile filaments of smooth muscle cells could be observed. The diameter of these actin filaments was about 5 nm. Another type of filaments, the intermediate filaments was found to have a diameter of 10 nm. The structure of dense bodies, dense bands and cavaeolae was similar to that of the smooth muscle cells of other organs. Three kinds of cell junctions were distinguished by electron microscopy in this study. These were the gap junctions, the intermediate junctions and the finger-like processes.

Key Words: Smooth Muscle, Mice, Ultrastructure, Airway.