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MORPHOLOGICAL ELEMENTS OF CONTROL OF SMOOTH MUSCLE ACTIVITY IN THE FROG STOMACH: AN EM STUDY

R. GÁBRIEL

Department of Zoology, Attila József University
H—6701 Szeged, P. O. B. 659. Hungary

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Abstract

The elements of the myenteric plexus, the interstitial cells of Cajal and the entero-endocrine cells of the frog stomach were studied with a transmission electron microscope. The profiles of the plexus and the fine structural features of the neuromuscular junction were described. The interstitial cells were identified at the ultrastructural level: a small amount of smooth endoplasmatic reticulum was observed in their cytoplasm. A few entero-endocrine cells were found in the epithelial layer. Their ultrastructure was similar to that in other vertebrates. The possible action of these elements on the contraction of the musculature suggests a multifunctional influencing of peristalsis: namely (1) a direct action of the nerves on the muscles; (2) changes in the pacemaking activity of the interstitial cells; (3) local systemic action of serotonin and peptides from entero-andocrine cells on the musculature.
Key words: frog stomach-myenteric plexus- interstitial cellsentero-endocrine cells-ultrastructure.

Introduction

Since the first reports on the innervation of the frog alimentary canal (BOTAZZI, 1899; CAJAL, 1902; DIXON, 1902; GAUPP, 1899) a considerable number of papers have dealt with this topic (e. g. BOYD et al., 1964; CAMPBELL, 1969; COLE, 1926; GUNN, 1951; HIRT, 1934; RASHID, 1972; READ and BURNSTOCK, 1968, 1969; YÜH, 1931). ANDERSON and CAMPBELL (1984) provided evidence of the presence of serotonin in the neurons of *Bufo* gut. As regards the frog stomach innervation, only classical morphological data are available (CAJAL, 1902; COLE, 1926; DIXON, 1902; GUNN, 1951; WONG et al., 1971). The gross morphology of the myenteric plexus of the frog stomach was recently described (GÁBRIEL et al., 1987) established by means of NADH-diaphorase histochemistry. However, the electron microscopic features of these elements have not been described to date. There is strong evidence that, besides the myenteric nerve elements, the interstitial cells of Cajal (ICCs) and the entero-endocrine cells (EECs) should also be taken into consideration as possible sources of substances able to influence the contraction activity of smooth muscle cells (BUCHAN et al., 1983; CRIM and VIGNA, 1983; FAUSSONE-PELLEGRINI, 1987; HOLMGREN, 1985; MIKKELSEN et al., 1988; ROMBOUT and REINECKE, 1984). The aim of our study was to describe the ultrastructure of elements which may influence the contraction activity of the smooth muscle cells in the frog stomach.

Material and methods

Six adult frogs (males and females) were used in this study. The stomachs were fixed either by immersion after distension with Krebs solution (for 3hr) or by transcardial perfusion followed by immersion (for 3hr). The fixative contained 2% parafomaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (PB) at pH 7.4 in both cases. Small tissue blocks were cut from the stomachs and postfixed in 1% OsO_4 (in 0.1 M PB) for 1hr. Blocks were then dehydrated in ascending series of ethanol, contrasted „en bloc" in 70% ethanol saturated with uranyl acetate, and embedded in Durcupan ACM resin. Ultrathin sections were cut with a Reichert OM U2 ultramicrotome and contrasted with lead citrate after REYNOLDS (1963). Preparations were viewed and photographed in Jeol EM 100B and Tesla BS 540 electron microscopes.

Results

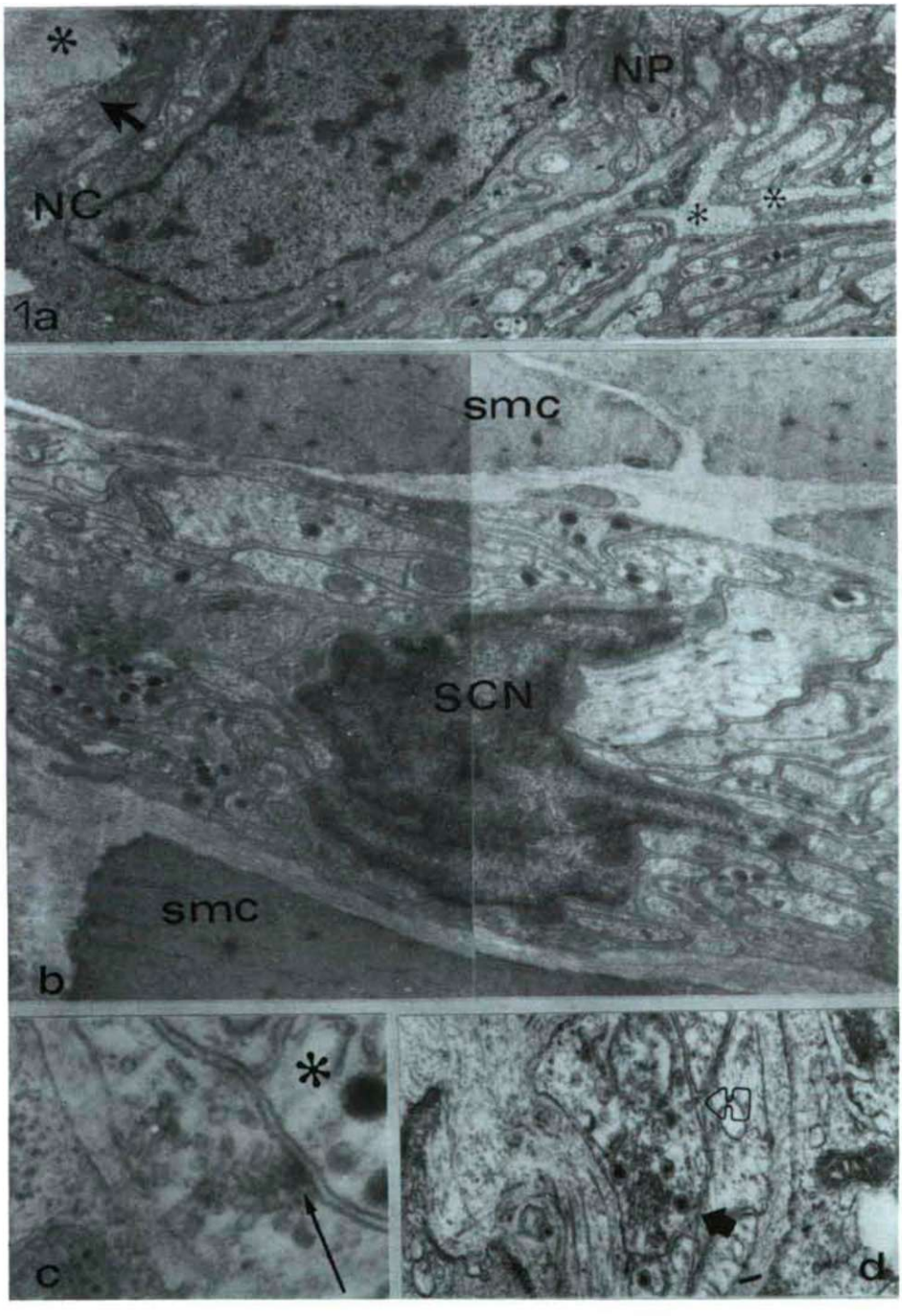
Large numbers of nerve processes and some nerve cells were revealed in the myenteric neuropile close to the outer surface of the stomach (Fig. la). Nerve cells were embedded into the main strands of the plexus. The profiles in the neuropile often contained large granulated vesicles (LGVs). The nerve cells and the surrounding neuropile were covered with Schwann-sheath and a collagen field. The interstitium was rich in collagen on the mesenteric side of the plexus. Large nerve bundles left this neuropile region to innervate the musculature of the stomach (Fig. 1b). 20—100 axons covered with Schwann-sheath were seen among the muscles. In some cases, synaptic contacts were observed between these axons (Fig. 1c), where the presynaptic density was prominent, while the postsynaptic thickening was lacking. The presynaptic profile contained clear vesicles while the postsynaptic one LGVs. Another type of contact seemed to be exocytosis between neighbouring profiles to release the transmitters from LGVs (Fig. 1d). Nerve bundles running to the muscles contained mainly 2 types of vesicles: small agranular vesicles (AGVs) and LGVs (Fig. 2a). Profiles with flattened vesicles (FVs) and also with dense-core vesicles (DCVs) were observed very rarely (Fig. 2b). Axons running parallel to the muscles sometimes established large surface close contacts, which were more than 1 μm long (Fig. 2c). As regards the junctional gap between the axolemma and the muscle cell membranes at the sites of close contacts, this did not exceed 20—40 nm

Fig. 1.a) Myenteric neuron (NC) and the surrounding neuropile (NP). The lateral side of this compact structure (arrow) is covered by Schwann cell processes. A collagen field (asterisk) can be seen between the neighbouring nerve bundles. Magnification: 7000x.

1.b) Schwann cell and axon profiles among the smooth muscle cells (smc). Some of the profiles contain large granulated vesicles (LGVs, arrows). Note the strongly heterochromatic structure of the Schwann cell nucleus (SCN). Magnification: 18000x.

1.c) Axo-axonic synapse in the deep neuropile. Only the presynaptic density (arrow) is conspicuous. The postsynaptic profile (asterisk) contain LGVs. Magnification: 32000x.

1.d) Exocytosis from a LGV. Note that the extracellular space also contains dense material (open arrow), which is probably a residue of a previous exocytotic process. Magnification: 32000x.


NP
NC
1a
smc
SCN
smc
b
c
d

(Fig. 3a), mainly where LGVs or mixed profiles established them. The release of LGVs took place with exocytosis (Fig. 3a, insert). When the contacting profiles contained AGVs only the junctional gap was approximately 100—150 nm wide (Fig. 3b).

Among the muscles, ICCs were also present (Fig. 4a), generally on the inner side of the circular muscles. Their nuclei were relatively rich in chromatin and their cytoplasm contained secretory granules of different sizes. Rough endoplasmatic reticulum cisternae, mitochondria, glycogen granules were present here (Fig. 4b).

Entero-endocrine cells were demonstrated among the epithelial cells of the frog stomach relatively frequently. They had a beanshaped nucleus and a large number of secretory granules were found in the narrow cytoplasmatic area (Fig. 4c).

Discussion

In the vertebrate alimentary canal, 3 different structures should be considered which are able to influence the contraction of the gut musculature: the nerves of the myenteric plexus, the ICCs and the EECs.

The myenteric plexus is well developed in the frog stomach and situated close to the outer surface of this organ (GÁBRIEL et al., 1987). This position is not unique among the vertebrates, for the same arangement has been observed in the gizzard of birds (ÁBRAHÁM, 1936; GABELLA and HALASY, 1987; IWANOW, 1930). The myenteric nerve cells are relatively small and not so densely packed as in higher vertebrates (GABELLA, 1987; GABELLA and HALASY, 1987; GÁBRIEL et al., 1988). The neuropile region is not so extensive as in mammals (GABELLA, 1979), and the synapses are scarce. These characteristics are typical for the lower vertebrates (BENEDECZKY et al., 1987; HALASY et al., 1988; TAXI, 1982).

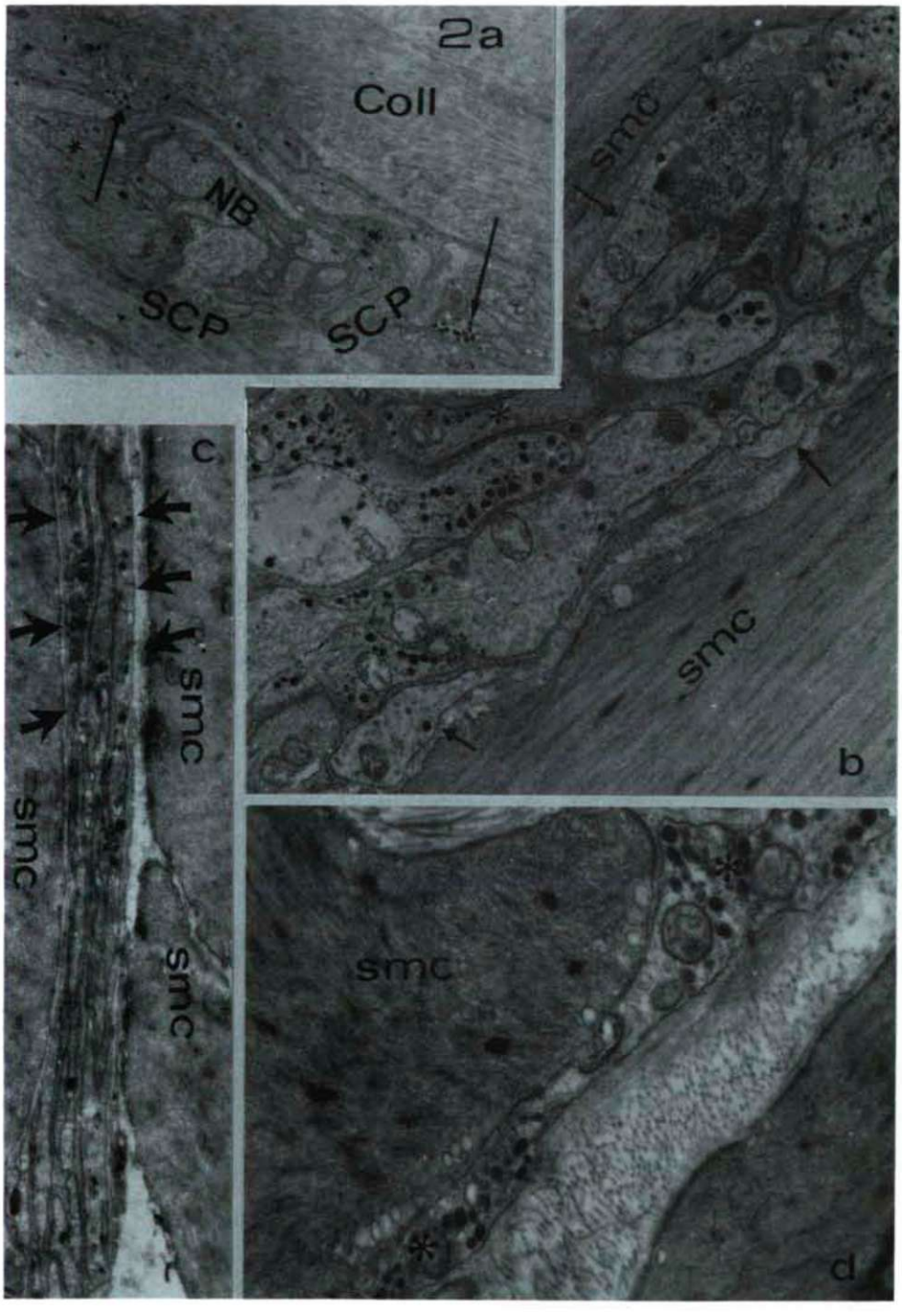
Axo-axonic synaptic contacts have been observed in the stomach, as well as in the large intestine of frog (GÁBRIEL et al., accepted for publication). Thus, this kind of synapse may be consequently present throughout the full length of the alimentary canal. Besides the synaptic contacts, another important form of communication between the different elements is non-synaptic transmitter release. The exocytosis of transmitter molecules from the DCVs and LGVs has mostly been proved with the

Fig. 2.a) Nerve bundles (NB) surrounded by collagen field (coll) among the smooth muscle cells (smc). Nerves are covered by Schwann cell prrocesses (SCP). Arrows: LGV-containing profiles; asterisks: profiles with clear vesicles. Magnification: 3400x.

2.b) Nerve plexus between smooth muscle cells (smc). Flattened vesicle (arrowhead) and dense-core vesicle (asterisk)-containing profiles are also visible. Note that a considerable proportion of the profiles are free of Schwann-sheath (arrows). Magnification: 20000x.

Fig. 2.c) Thin axons in a narrow space between smooth muscle cells (smc). Relatively long close contacts (arrows) are visible. Magnification: 18000x.

2.d) An axon with double active site (asterisks) establishes close contact with a smooth muscle cell (smc). Both vesicle accumulations contain agranular vesicles and LGVs. Magnification: 30000x.


2a
Coll
NB
SCP
SCP
smc
smc
b
c
smc
smc
smc
smc
d

TARI method (BENEDECZKY and HALASY, 1988; BUMA et al., 1984), but also described under normal EM fixation conditions (ZHU et al., 1986).

As concerns the possible transmitters, acetylcholine is known to play this role in the frog stomach (RASHID, 1972; WONG et al., 1971), and also the presence of γ-aminobutyric acid has been proved (GÁBRIEL and ECKERT, 1989). BURNSTOCK (1972) postulated the purinergic hypothesis, which was also discussed by SNEDDON et al. (1973). However, morphological investigations have not yet been performed in this respect. No evidence has been obtained on the presence of peptide transmitters so far, but the axon profiles with mixed vesicle content suggest cotransmission (BURNSTOCK, 1976, 1978; HÖKFELT et al., 1977; SCHULTZBERG et al., 1980). Because of the great number of muscle contacting LGV and mixed profiles, the role of peptide/purine neurotransmission is of significance in the frog stomach. The most important peptide transmitter candidate is Substance P, which is known to have a strong stimulating effect on muscles and also on other nerve endings, in both lower (BJENNING and HOLMGREN, 1988; JENSEN et al., 1987) and higher vertebrates (BARTHO and HOLZER, 1985; SMITH et al., 1988).

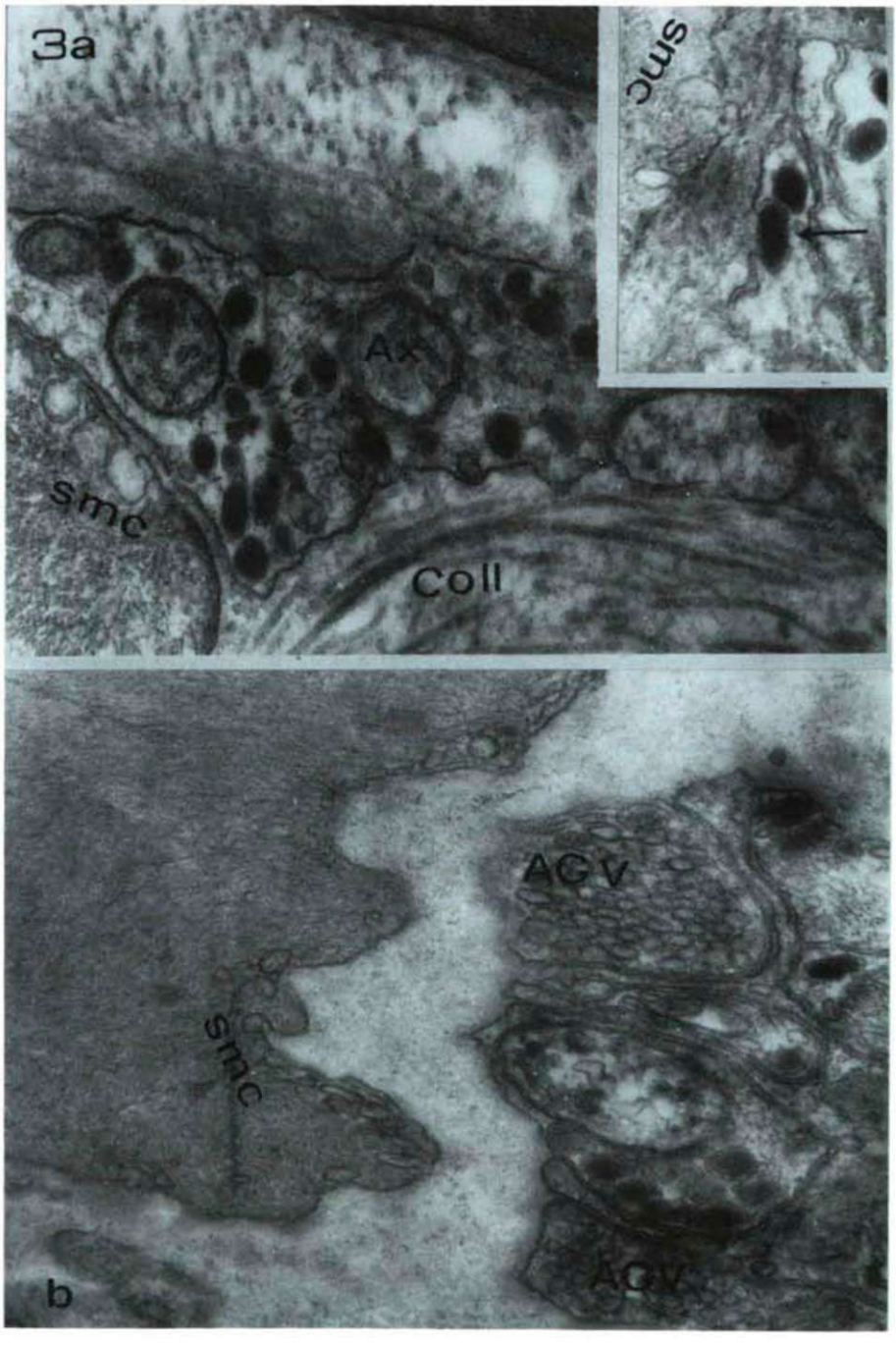
The first description of ICCs was reported by CAJAL (1893). Their presence in the frog stomach muscular layer was revealed by COLE (1925). Descriptions at the EM level are available on the ICCs of different mammals (FAUSSONE-PELLEGRINI, 1984, 1987), but not those of the frog stomach. The general view of ICCs in the frog stomach is not basically different from that for mammals. The smaller amount of smooth endoplasmatic reticulum of the frog ICCs may be considered to be the only main morphological difference. The role of these cells remains obscure almost a century after CAJAL, although some authors (FAUSSONE-PELLEGRINI, 1984, 1985; STACH, 1972; TAYLOR at al., 1977) regard them as an element of a functional chain: nerve endings-ICCs-smooth muscles. The ICCs might act as pacemakers which are controlled by nerve endings. Further studies are needed to ascertain the exact role of these cells.

EECs are known to contain serotonin and different peptides (CCK-gastrin, bombesin, somatostatin) in the lower vertebrates, too (CRIM and VIGNA, 1983; ROMBOUT and REINECKE, 1984). The presence of a high amount of secretory granules in these cells suggests that an intensive peptide-secreting process occurs here. The same granules may also contain serotonin, because PELLETIER et al. (1981) poved the presence of serotonin and a peptide (Substance P) within one DCV. These substances might act through the local circulatory system and also by diffusion across the connective tissue layer. The seasonally different effects of

Fig. 3.a) The junctional gap is not more than 20—50 nm when the contacting profile (Ax) includes LGVs. Coll: collagen, smc: smooth muscle cell. Magnification: 32000x.

Insert: exocytosis (arrow) in the close proximity of a smooth muscle cell (smc). Magnification: 64000x.

3.b) When the contacting profile contains agranular vesicles (AGV), the junctional gap is slightly more than 100 nm. smc: smooth muscle cell. Magnification: 60000x.


3a
smc
Ax
smc
Coll
AGV
smc
AGV
b

serotonin on the frog stomach were clarified by SINGH (1964); he found that this substance has an excitatory effect in spring and an inhibitory effect in other seasons.

In conclusion, the 3 different structures described here influence the smooth muscle activity in a complex manner, in 3 different ways: (1) nerves may act directly on the smooth muscle cells; (2) the ICCs are able to change their own pacemaker activity (also influenced by nerves?); and (3) the active substances of the EECs (serotonin and peptides) might act through the local circulatory system.

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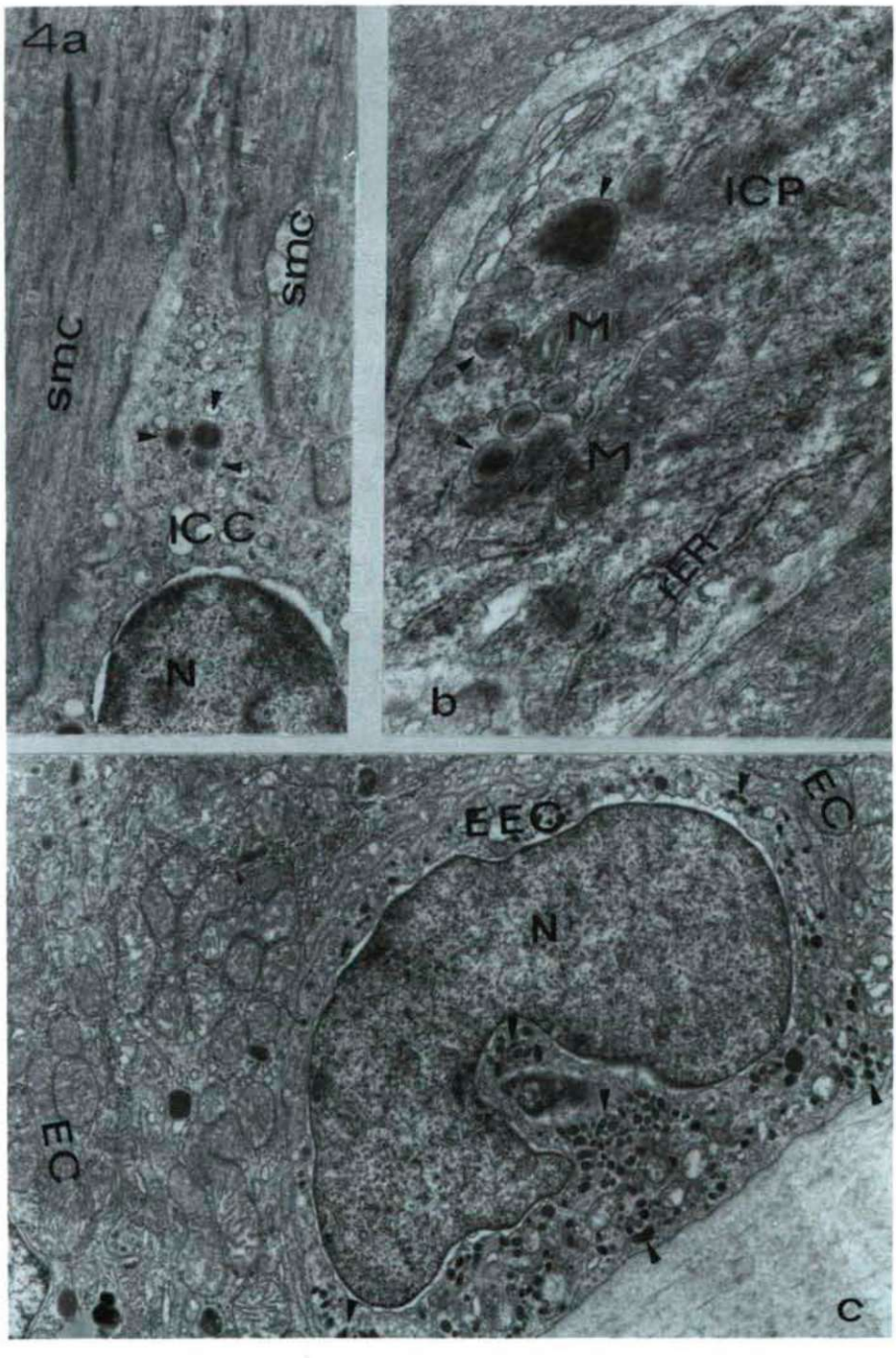
Fig. 4.a) ICCs are often present between the smooth muscle cells (smc). The nucleus (N) is rich in chromatin, and the cytoplasm contains secretory granules (arrowheads). Magnification: 34000x.

4.b) The ICC processes (ICP) form knobs between the muscles: mitochondria (M), rough endoplasmatic reticulum (rER) and granules (arrowheads) are also present. Magnification: 34000x.

4.c) EEC among other epithelial cells (EC). A bean-shaped nucleus (N) and large amount of secretory granules (arrowheads) are characteristic of it. Magnification: 18000x.


4a
smc
smc
ICC
N
ICP
M
M
SER
b
EEC
EC
N
EC
c

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