NEXUSES BETWEEN AREAS OF THE SURFACE MEMBRANE OF THE SAME ARTERIAL SMOOTH MUSCLE CELL

TAKASHI IWAYAMA. From the Department of Anatomy, Faculty of Medicine, Kyushu University, Fukuoka, Japan

INTRODUCTION

Specialized areas of close apposition (nexuses) are found between the smooth muscle cells of various organs (Dewey and Barr, 1962, 1964), including blood vessels (Cliff, 1967; Verity and Bevan, 1968; Devine and Simpson, 1968). The nexus may appear as either a tight junction, with apparent membrane fusion, or a gap junction, possibly depending on the method of fixation (Brightman and Reese, 1969; and compare Cobb and Bennett, 1969 *a* with Uehara and Burnstock, 1970). These structures may be important in the conduction of electrical events which is known to occur between smooth muscle cells of various organs (Bozler, 1938; Tomita, 1967; Barr et al., 1968; Abe and Tomita, 1968). In addition, conduction between arterial smooth muscle cells is suggested by the successful use of the sucrose-gap method to record from this tissue (Keatinge and Richardson, 1963; Keatinge, 1964).

In the present work, intercellular junctions in arterial smooth muscle have been examined. Junctions between areas of the surface membrane from the same cell are also described and some implications of this finding are discussed.

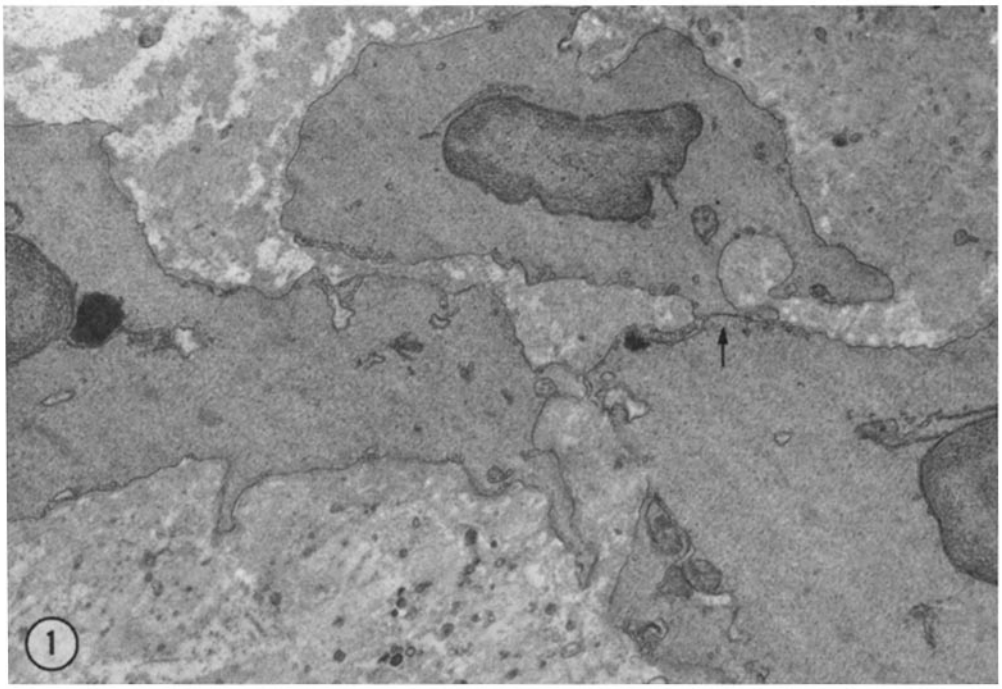
MATERIAL AND METHODS

The material examined was from the human aortic arch, a specimen of which was removed from a 22 year old patient suffering from congenital coarctation of the aorta. The tissue was fixed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) for 1 hr. It was then placed in 4% glutaraldehyde for 1 hr followed by a further hour in the osmium tetroxide solution. Between the fixations, the specimen was washed in several changes of distilled water over a period of 3 min. The fixed tissue was stained in a 2% aqueous solution of uranyl acetate for 1 hr, gradually dehydrated in acetone, and embedded in Araldite. All the procedures were carried out at room temperature. Thin sections were cut with a Huxley Ultra-Microtone (Cambridge Instrument Co., Inc., Ossining, N.Y.), stained with lead citrate, and examined with an Hitachi HU 11 b electron microscope at 75 kv.

RESULTS

Smooth muscle cells, often separated by large areas of the extracellular matrix containing collagen and elastin, were sparsely distributed in the media of the aorta (Fig. 1). Generally, the smooth muscle cells were of irregular shape and they often exhibited prominent processes and invaginations of the surface membrane (Fig. 1). Some of them contained electron-opaque bodies. The external lamina of the muscle cells was inconspicuous (Figs. 2, 3). Nexuses between the membranes of adjacent cells were often observed (Figs. 1, 2). They were generally observed between processes of the cells and took the form of either peg-and-socket or planar contacts.

Similar junctions were also seen between areas of the membrane of processes arising from the one cell (Figs. 3, 4); in the junctional areas, the cell membranes lay parallel to each other with a clearly demonstrable gap between the outer leaflets of the unit membranes (Figs. 2, 4). The size of the gap was similar for all junctions—20–30 A across.



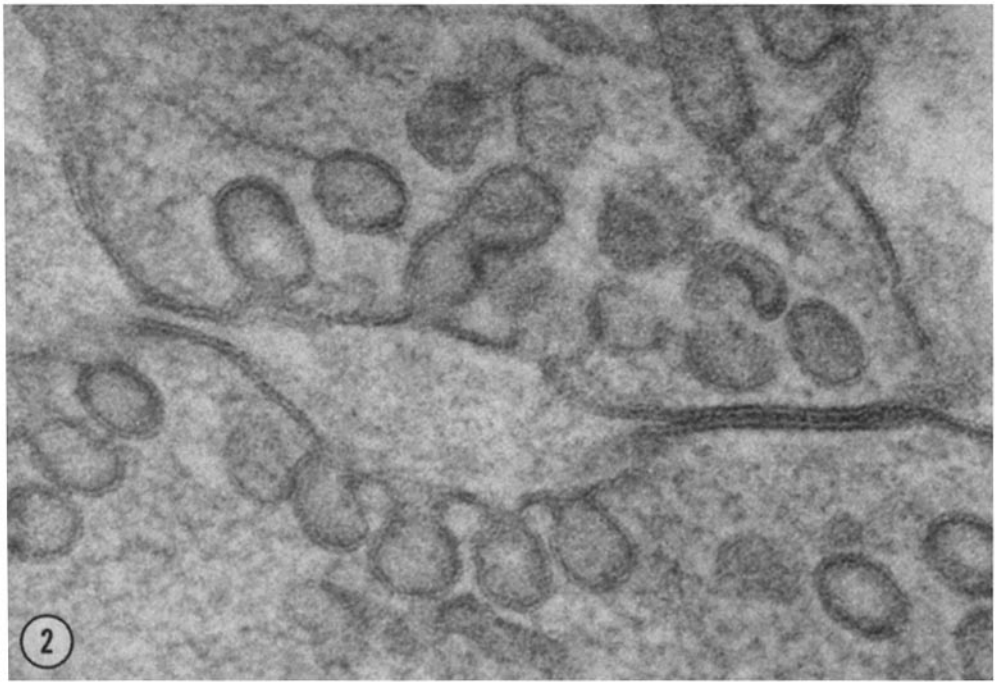


FIGURE 1 Irregularly shaped smooth muscle cells of the human aortic arch. Close apposition is observed between small areas of adjacent cells (arrow), while the other parts are separated by large areas of the extracellular matrix. Some smooth muscle cells contain electron-opaque bodies. × 6500.

FIGURE 2 A nexus between two smooth muscle cells. A small, regular gap (about 20 A) is present between the outer leaflets of the unit membranes. × 180,000.

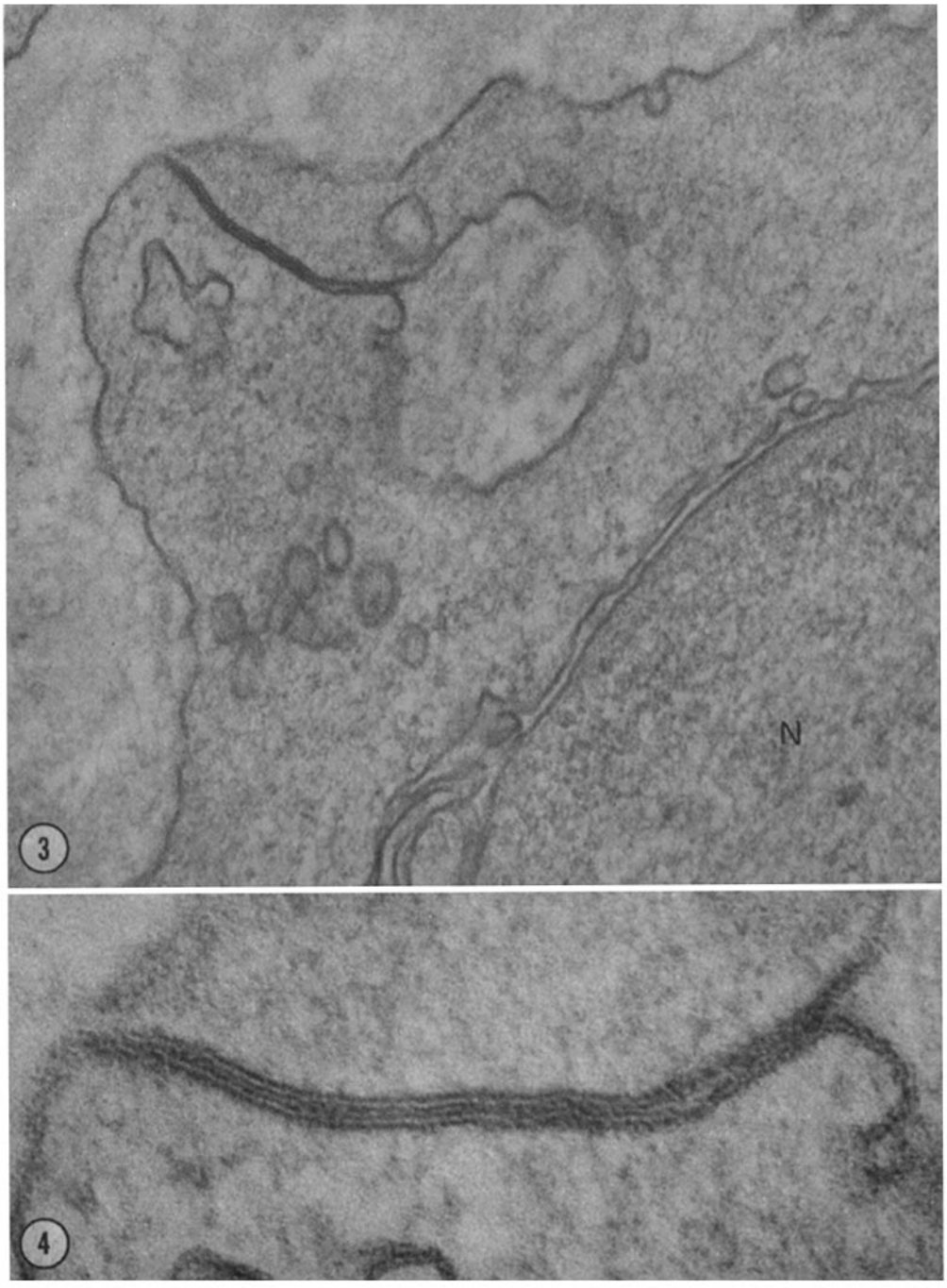


FIGURE 3 Processes from a single smooth muscle cell coming together to form a nexus. Note the indistinct basement membrane of the muscle cell. *N*, nucleus. × 90,000.

FIGURE 4 A high-power electron micrograph of the area of the nexus in Fig. 3. Note the clear gap between the areas of the membrane at the junction. × 300,000.

DISCUSSION

Nexuses between adjacent smooth muscle cells are thought to be low-resistance areas for electrical conduction between the cells (see Dewey and Barr, 1964, 1968; Barr et al., 1968; Cobb and Bennett, 1969 *a*; Furness, 1970). Whether these junctions are formed in an ordered manner, or whether they merely arise randomly where the surfaces of the membrane are sufficiently closely apposed, is not known. The arterial smooth muscle cells of the tissue examined in the present work had long processes, making possible a direct contact between different parts of the surface of the one cell. Nexuses were often observed where processes of a single cell were in mutual contact, suggesting that, at least in this tissue, nexuses will form wherever sufficiently close contact is made between muscle cells. However, functional differences, not indicated by their superficial similarity, may exist between these nexuses and those linking different cells. Similar junctions between areas of the membrane of the one smooth muscle cell have been observed in guinea pig vas deferens (unpublished observations). There appeared to be a reduction in the amount of basement membrane material around the muscle cells of this tissue. This paucity of external lamina may facilitate the formation of junctions between the cell membranes by permitting more intimate contact.

Recently, certain intercellular junctions have been proven to have small gaps (Revel and Karnovsky, 1967; Goodenough and Revel, 1970). The appearance of the junctions differed with different preparative techniques (Brightman and Reese, 1969; Cobb and Bennett, 1969 *b*). The existence of a small gap between the outer leaflets of the unit membranes has also been shown in junctions between smooth muscle cells after block staining with uranyl acetate (Revel et al., 1967; Uehara and Burnstock, 1970), and with high voltage electron microscopy (Hama and Porter, 1969). Intercellular junctions of blood vessels have been reported as fused junctions by Cliff (1967). However, after uranyl staining in block, the present work showed gap, rather than five-layered, junctions in apposed areas of the surface membrane of the same smooth muscle cells, and also of adjacent smooth muscle cells in the human aorta.

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