GAP JUNCTIONS IN ARTERIAL ENDOTHELIUM

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INTRODUCTION

Tight junctions are well characterized membrane specializations in vascular endothelium. These structures seal the interspace between adjacent endothelial cells, either completely, as in cerebral vessels (10), or incompletely, as in striated and cardiac muscle capillaries (8, 19) as well as in non-cerebral arteries (4, 5). Gap junctions in vascular endothelium have not been described in the literature as far as we know. Extensive membrane appositions were recently reported in the endothelium of rat thoracic aorta, but these structures were interpreted as tight junctions (16). The present study, utilizing uranyl-treated and lanthanum-permeated tissue preparations, identified gap junctions in the endothelium of selected arterial regions. The possible functional significance of the finding is discussed.

MATERIALS AND METHODS

Endothelial cells were examined in segments of thoracic aorta, carotid artery, abdominal aorta, external iliac artery, in small arteries in the vicinity of the aorta and carotid artery, as well as in intramural coronary arteries. The tissues were obtained from 12 male Sprague-Dawley rats weighing 300–400 *g* each.

Preparation for Electron Microscopy

Fixation was initiated by perfusion of Karnovsky's fixative (7) containing 1% freshly prepared paraformaldehyde and 1.25% purified glutaraldehyde in 0.1 N sodium cacodylate buffer (pH 7.4), with 5% sucrose (final osmolality: ~750 mosmol). Perfusion was started under ether anesthesia through the left ventricle of the heart, and maintained for 10 min at 120 mm Hg pressure while allowing the fixative to escape through the vena cava inferior. Segments from the large arteries cited above, as well as pieces from the right ventricular myocardium were then removed, minced, and immersed in a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde with the same buffer (final osmolality: ~900 mosmol) for 4–6 h at room temperature. The tissues were washed overnight at 4°C in 0.1 N sodium

cacodylate buffer (pH 7.4) containing 11.25% sucrose (final osmolality: ~380 mosmol); this was followed by fixation for 90 min at 4°C with 1% OsO_4 in Palade buffer (pH 7.4) containing 4.9% sucrose (final osmolality: ~430 mosmol). Parts of the tissues from each specimen were then treated for 2 h at 4°C with 2% uranyl acetate (8) in sodium hydrogen maleate-NaOH buffer 0.05 M (pH 6.0), followed by a short washing in maleate buffer (pH 5.2); all tissues were then dehydrated in graded ethanols and embedded in Epon 612. For light microscopy, 1 μm sections were stained with toluidine blue in borax. For electron miscroscopy, silver-to-gray sections were cut with a diamond knife on an LKB III microtome and examined, either unstained or after lead citrate staining (18), with a Philips EM-300 microscope.

Permeation Studies with Lanthanum

Tissues were permeated with fixatives containing neutralized lanthanum (11), which was prepared by dissolving 4% lanthanum nitrate in water and slowly adding 0.01 N NaOH to pH 7.7. This solution was

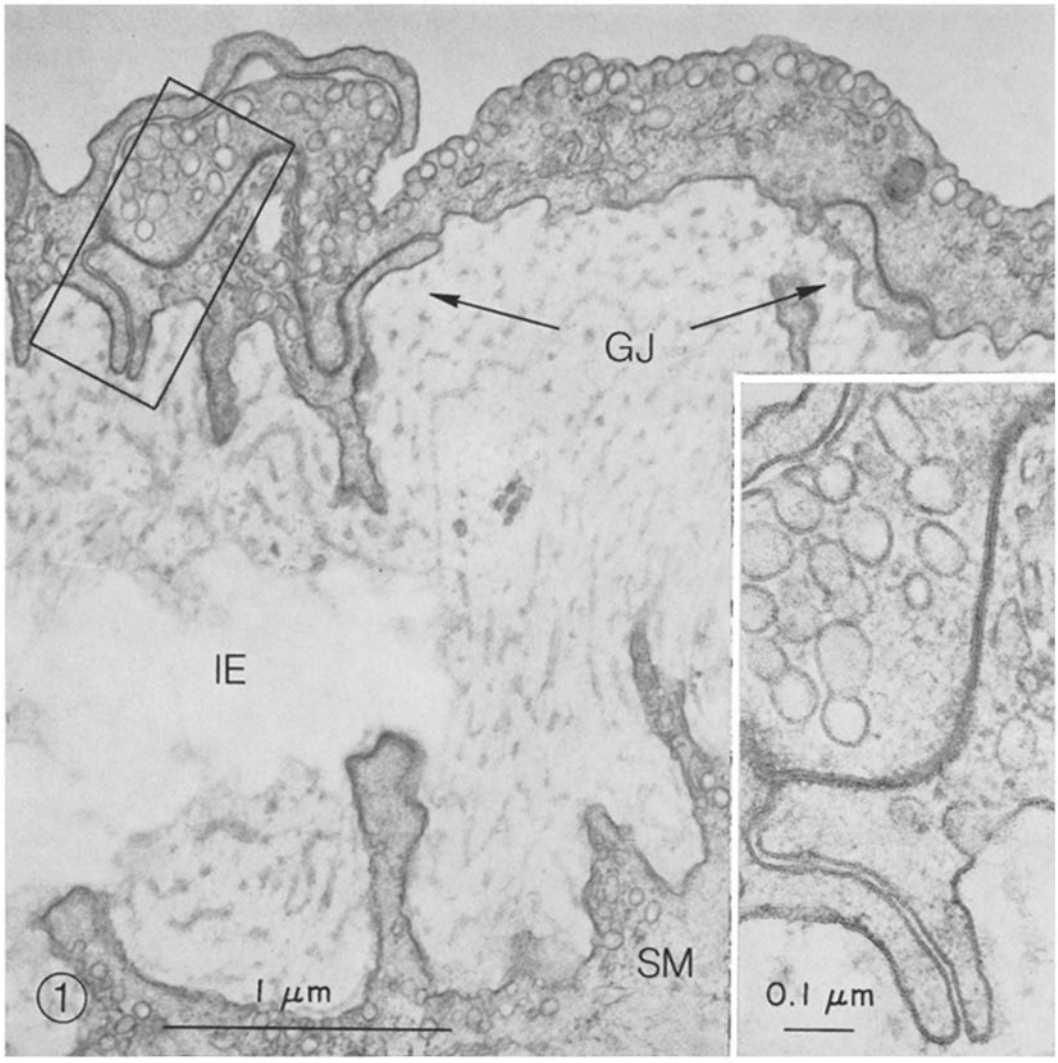
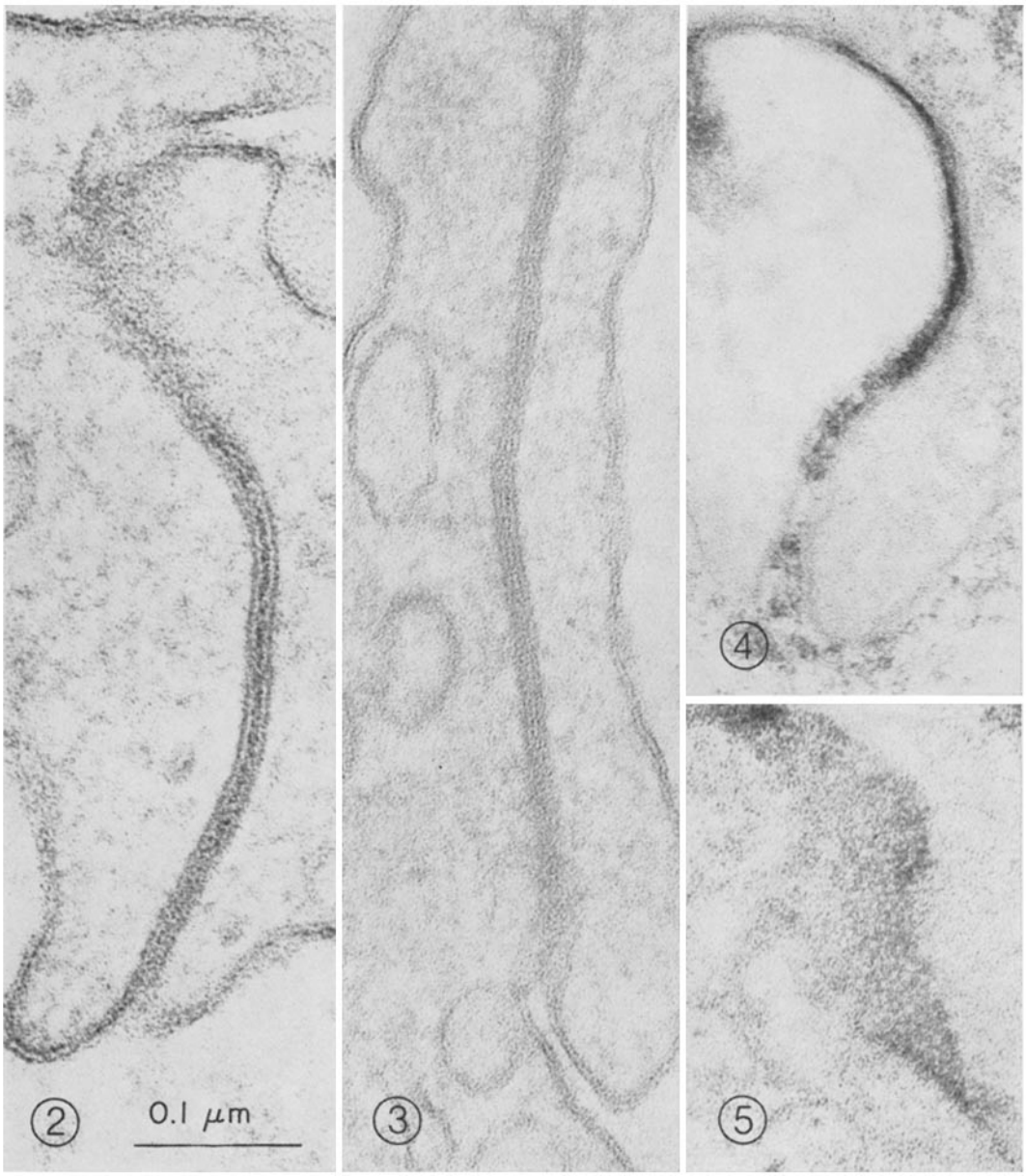


FIGURE 1 Detail from rat carotid artery. Large gap junctions (*GJ*) between endothelial cells appear as darkly staining lines along intercellular clefts. *IE*, internal elastica; *SM*, smooth muscle cell. × 38,000; *inset*, × 90,000.

added to equal volumes of aldehyde fixative prepared in such a way that the final concentration of aldehyde was equal to that specified above, both for perfusion and for immersion fixation. Tissues were postfixed with a 1.1 mixture of 2% OsO_4 in sodium cacodylate buffer and neutral lanthanum.

OBSERVATIONS

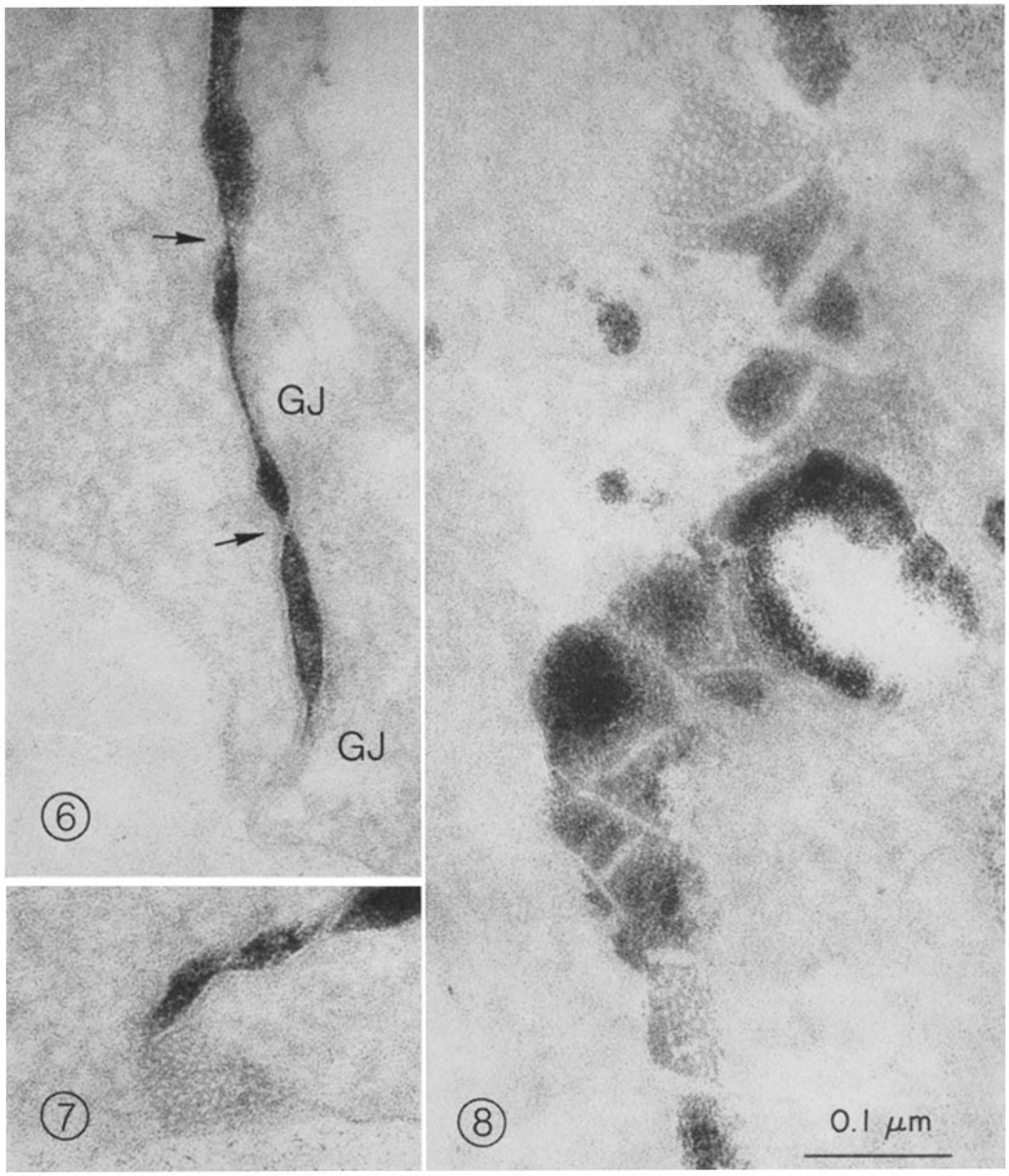
In tissues treated with uranyl acetate en bloc, the individual leaflets of the endothelial plasma membranes were clearly defined. Intercellular clefts between adjacent endothelial cells were quite di-



FIGURES 2–5 Gap junctions from carotid artery endothelium. Lead staining on uranyl acetate-treated preparation renders the gap dense between the apposed cell membranes (Fig. 2). Uranyl acetate treatment alone, however, visualizes a constant 20–40 Å gap between the external membrane leaflets, forming a septilaminar structure (Fig. 3). Lanthanum permeates the 20–40 Å gap (Fig. 4) and reveals regularly packed subunits in *en face* view of the gap junction (Fig. 5). × 230,000.

verse in length and configuration and varied in width from 100 to 200 Å. At one or more points of the clefts, adjacent plasma membranes were closely approximated, forming cell junctions. Two distinct types of junctions were identified between adjacent endothelial cells in all arterial regions examined, both types often being present along the same intercellular clefts:

(*a*) Punctate contacts or fusions of the outer leaflets of the apposed endothelial plasma mem-



FIGURES 6 and 7 Interendothelial clefts from small artery adjacent to the carotid. Small gap junctions (*GJ*) in transverse (Fig. 6) and *en face* (Fig. 7) views. The basally located gap junction seen in Fig. 6 is only partially permeated by lanthanum. Arrows indicate nonpermeated points or lines of tight junctions. × 200,000.

FIGURE 8 *En face* view of intercellular cleft from lanthanum-permeated coronary artery endothelium. Subunits of gap junctions are outlined by white lines of tight junctions. × 200,000.

branes, forming small pentalaminar foci. In lanthanum-treated tissue preparations, these structures appeared as nonpermeated points in transversely cut intercellular clefts (Fig. 6) and as single or branching white lines in *en face* view (Figs. 7 and 8), and were identified as *tight junctions*. Their permeability characteristics and distribution in the arterial endothelium have been described in detail elsewhere (4, 5).

(*b*) Extensive membrane appositions forming large septilaminar foci. These structures were readily recognized even at low magnification as darkly staining lines with a long, gently undulating contour (Fig. 1). Lead citrate staining of sections prepared from tissue treated with uranyl acetate en bloc rendered the gap between the cell membranes dense and gave the junction the appearance of a pentalaminar structure (Fig. 2); the central density, however, was wider than that found in the true pentalaminar foci. Uranyl acetate treatment alone visualized a constant gap of 20–40 Å between the external leaflets of the apposed plasma membranes along the entire length of these junctions, clearly unmasking the septilaminar structure (Fig. 3). When neutral lanthanum was used to delineate the extracellular space, it permeated the 20–40 Å wide gap between the external membrane leaflets (Figs. 4 and 6), and revealed regularly packed subunits in *en face* view (Figs. 5, 7, and 8), identifying these structures as *gap junctions*. In *en face* view, subunits of gap junctions were often outlined by nonpermeated lines of tight junctions (Figs. 7 and 8). While gap junctions did not occur in each intercellular cleft in the plane of a section, they were numerous in the endothelium of all arterial regions examined, particularly in coronary arteries.

DISCUSSION

Whereas tight junctions form local permeability barriers between cells, gap junctions are considered as low resistance pathways for intercellular communication (1, 3); they are widely implicated in cell-to-cell transfer of ions (ionic or electrotonic coupling) (2, 9, 13) and in cell-to-cell transfer of cellular metabolites (metabolic coupling) (2). In this regard, the identification of gap junctions in arterial endothelium may suggest a new aspect of the arterial endothelium that serves as the structural basis for a synchronized function. Gap junctions have been reported to occur between arterial smooth muscle cells (6), similar to those found in visceral smooth muscle (12, 17). Myoendothelial junctions, described previously as tight junctions in arterioles and precapillary sphincters (14), do occur in large arteries as well; in the light of the present study, the nature of these junctions remains to be clarified. If gap junctions at such location are shown to exist, it would suggest not only that, in arteries, endothelial cells are coupled cells, but also that the entire endothelial and smooth muscle apparatus do form a coupled system. This hypothesis correlates well with the previous suggestion that receptor sites are localized on the surface of endothelial cells and that endothelial cells may transmit some signal to smooth muscle cells (14, 15).

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