ENDOTHELIAL-SMOOTH MUSCLE RELATIONSHIP

ON THE FUNCTIONAL IMPORTANCE OF VASCULAR ENDOTHELIUM FOR VASCULAR SMOOTH MUSCLE CONTRACTION

THESIS

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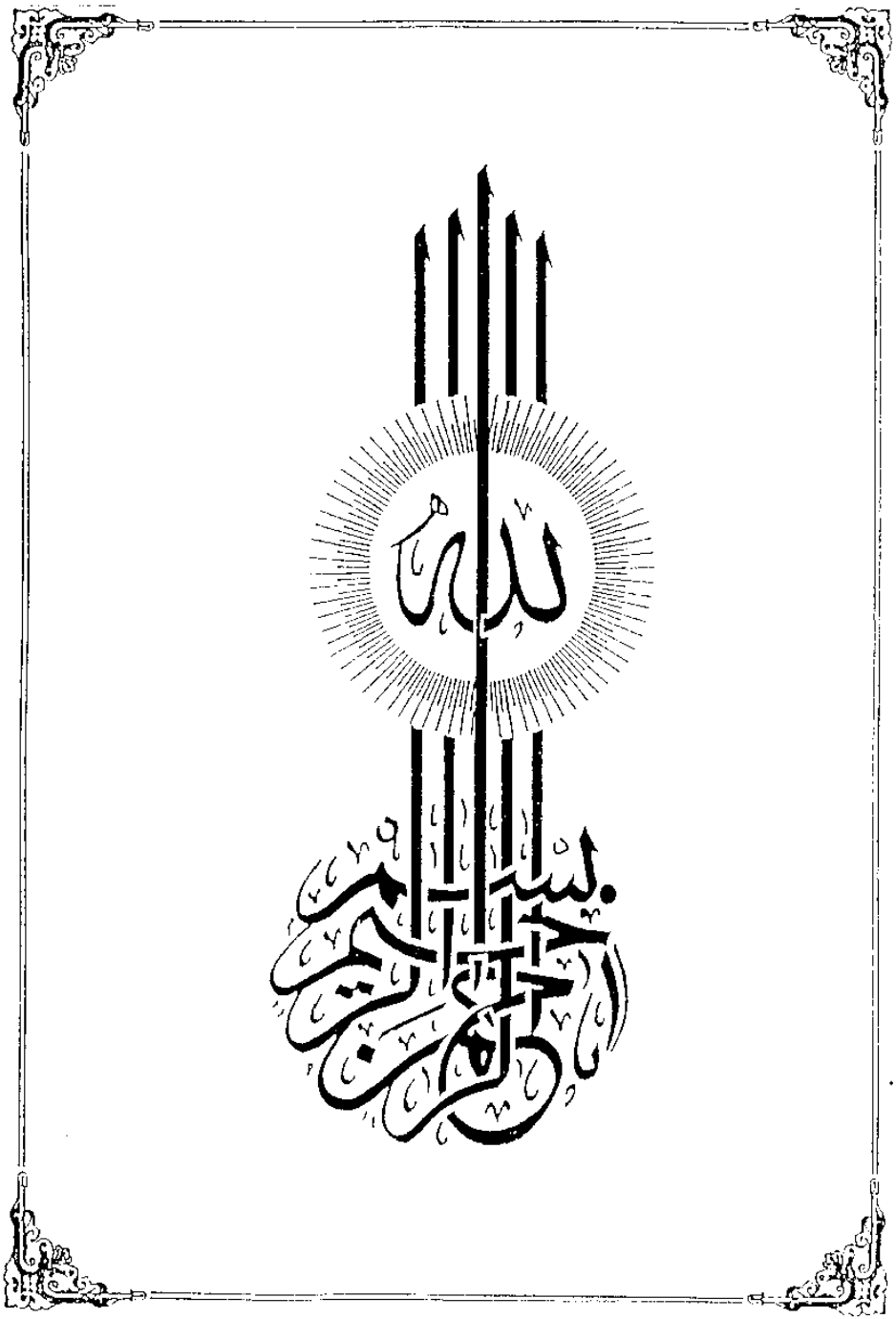

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INTRODUCTION

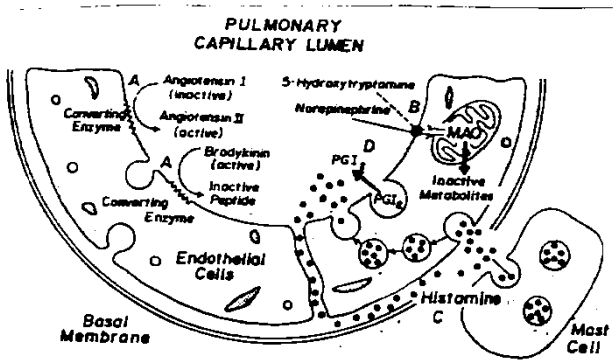

Fig.(1): The handling of bioactive substances by the endothelial cells. (A) Substances such as angiotensin I and bradykinin are transformed "on" the endothelium by converting enzymes , with resulting formation of active and inactive components, respectively. (B) Substances such as norepinephrine and 5-HT are actively taken up (O) and degraded enzymatically by monoamine oxidase (MAO). Other substances metabolically degraded by the endothelial cells include certain prostaglandins. (D) During allergic and anaphylactic reactions substances such as histamine can overflow into the capillary lumen. (C) It is likely that the endothelium can produce certain prostaglandin-like substances such as prostacyclin (PGI_2) and release them into the blood stream .

(Cited from Vanhoutte and De Mey, 1983 . Gen. Pharmac., 14 : 39 - 41) .

Thus , the early finding that cultured endothelial cells exhibit angiotensin converting enzyme activity (Hial et al., 1976) and possess the machinery for the synthesis of prostaglandins (Gimbrone and Alexander, 1975) can be considered the beginning of a trend towards a reevaluation of the role of endothelium and its relationship with other elements of the vessel wall and surrounding tissue . Besides their role in capillary transport and exchanges between blood and tissues, the endothelial cells are sites of active transformation of plasma constituents . Thus by activating angiotensin I and inactivating Bradykinin, Norepinephrine and 5-hydroxytryptamine (5-HT), the endothelial cells help determine the amounts of vasoactive substances which reach the vascular smooth muscle cells of the media (Shepherd and Vanhoutte, 1979) (Fig. 1) . Furthermore, it is now established that the endothelial cells are the major source of prostacyclin which is a potent vasodilator (Moncada and Vane, 1978) .

Moreover, in cultured endothelial cells, the presence of an array of receptors for various vasoactive compounds which traditionally have been associated with the phenomenon of contractility (Buonassisi and Venter, 1976) and the demonstration of adenyl cyclase in capillary endothelial cells which responded to catecholamines with increase cyclic AMP (Wagner et al., 1972) supports the hypothesis that endothelial cells may perform an important role in regulation of local circulation .

So, it may be presumed that the endothelium may be able to convert a hormonal stimulus into an electrophysiological impulse or a chemical signal that can be transferred to other cells of the vessel wall or to cells of surrounding tissue . The mechanism through which the stimulation of endothelial receptors may be passed on to the underlying smooth muscle is unknown . Junctional complexes have been described to occur between the endothelium and precapillary cell pericytes and between endothelium and arterial smooth muscle cells (Pease and Paule, 1960; Venity et al., 1965; Barr et al., 1968; Dahl, 1973; Rhodin, 1974 and Simionescu et al., 1975) . These junctions may represent sites of low resistance coupling (Bruns and Palade, 1968) or as has been shown in other biological systems (Gilula et al., 1972), a pathway for transfer of molecules between cells .

It is interesting to note that in arterioles, endothelial cells have been found to have foot-like processes that project into the smooth muscle cells to form a complex (Carlson et al., 1982)(Fig. 2). . Such an arrangement suggests that the intervening endothelial cells can act as transducers for selective passage of hormones from blood to smooth muscle (Rhodin, 1967) or as a pathway through which local metabolites and osmotically active substances might exert their action (Somlyo and Somlyo, 1968) .

Another possible functional role of the myo-endothelial junctions present in arterioles is that as intravascular pressure is elevated, it is likely that a portion of the endothelial cell content is extruded through the fenestration , deforming the vascular smooth muscle cell sarcolemma in the vicinity of the junction resulting in contraction . It is possible that the vascular smooth muscle contraction in response to the initial deformation would cause a lessening of the initial deformation thus providing a negative feedback which would limit the response to the pressure stimulus (Carlson et al., 1982) .

These findings have focussed attention on the potential importance of the endothelium in modulating the response of the smooth muscle of the blood vessel wall to local, humoral and nervous stimuli . Consequently , this study has been designed to outline the role of the vascular endothelial cells in mediating both excitatory and inhibitory responses of blood vessels and to determine endothelium-dependent differences between arteries and veins .

Ultrastructure Of Endothelium

The layer of the intima which is in direct contact with the circulating blood, is formed of endothelial cells that line the entire vascular system including the heart and lymphatic vessels and is 0.5 - 1.2 u in thickness (Asmussen and Kjeldsen, 1975 and Thureson-Klein, 1980) .

Electron microscopy of the luminal surface in several types of blood vessels, shows that the endothelial cells are fusiform and longitudinally oriented in the direction of blood flow (Figure 3) .

One specific organelle, first described in endothelial cells of small arteries in rat and man by Weibel and Palade (1964), is approximately 3 u long and 1 u thick containing small tubules embedded in a dense matrix . This provides a useful ultrastructure marker for arterial endothelium . These Weibel Palade bodies could be identified in cultured endothelial cells , and are common in vessels with diameter more than 30 um . In the Carp veins, Weibel Palade granules contain a primary catecholamine . It is suggested that these granules can take up and accumulate exogenous amines and that these mechanisms are resistant to reserpine (Iijina and Wasano, 1980) .

Typical cytoplasmic microtubules are present in most endothelial cells (300° A). They have a cytoskeletal function and play a role in translocation and release of various substances .

Microfilaments (50 - 100° A) are abundant in endothelial cells. They may have both supportive and contractile functions .

One important function of the endothelium is to mediate transport of substances between blood and tissues . Freeze fracture studies reveal a polar distribution of intramembraneous particles in the endothelial plasma membrane, which was interpreted to reflect a gradient for water and ion transport (Shaklai and Tavassoli, 1978) . The presence of caveolae was also demonstrated and might indicate high secretory and absorptive activities (Yamanato et al., 1976). These caveolae & pinocytotic vesicles are very rare in endothelial cells of capillaries from human placenta (Heinrich et al., 1976) and in small vessels and capillaries of brain, while they occupy one third of the cell volume in capillary endothelium of myocardium . The transport of substances in vesicles occurs from tissue to vessel lumen and vice versa, for example, acetylcholine esterase formed and released by neuronal cells, can be taken up by endothelial vesicles and transported to the luminal side, which could be visualized by cytochemical and ultrastructural techniques (Schubert and Kreutzberg, 1976) . On the other hand, lipoproteins ferritin, horse radish peroxidase are transported from plasma across the endothelium in vesicles (Stein and Stein , 1973) . Junctions between endothelial cells and between the endothelium and smooth muscle cells have been demonstrated (Fig. 4 , 5). Large arteries have a complex of occluding and communicating junctions (gap junctions)

Fig. (4): The vessel is lined by a continuous endothelium (E) the cells of which are connected at overlapping endo-endothelial junctions (j) . The subendothelial space is lined by basal lamina (bl) enclosing elastic fibers and collagen fibrils of which the internal elastic lamina (IEL) is composed . An endothelial protrusion (ME) breaks through the external lamina of the vascular smooth muscle cell to form a myoendothelial junction . v, Vesicle . X 27,000.

(Cited from Carlson et al;, 1982 . Microvasc. Res. , 24 : 123 - 141) .

Fig.(5): Electron micrograph of longitudinal section through arteriolar wall showing three types of intracellular junctions. Endo-endothelial junctions (j) often show large areas of overlapping cell processes. Myo-myocytic junctions (opposing arrows) almost always accomodate external lamina (el) material. Note also the location of dense bodies immediately adjacent to the junction . Myo-endothelial junctions (ME) frequently show rounded endothelial protrusions in well-fitting complementary invaginations in the overlying vascular smooth muscle cells. This morphology is typical of vessels fixed at normal intravascular pressure . db, Dense body ; L, lumen; MF_x, myofilament bundle in cross section; v, vesicle. X 44,000 .

(Cited from Carlson et al., 1982 . Microvasc. Res., 24 : 123 - 141) .

between the endothelial cells similar to those in arterioles. In comparison, vein have less complicated junctions which may be continuous for relatively long distances (Simionescu et al., 1976) . The junctions of capillaries and venules are usually short and blunt . The tight junctions are typical of brain arterioles, both between the endothelial cells and between endothelial and smooth muscle cells . On the other hand, fenestrated junctions are found in capillaries of adrenal gland . However , the size of fenestra differ from the cortex to medulla (Ryan et al., 1975) . In the cortex, the fenestrated endothelium allows the passage of cortical hormones to the circulation . In the medulla, the fenestrated endothelium allows the passage of catecholamines into the circulation . Dopamine-B-hydroxylase and chromogranin appear in the venous effluent when the adrenal medulla is stimulated, whereas the concurrently released ATP does not enter the vascular space .

Recent electron microscopic evidence suggests that the brain capillaries are innervated by adrenergic nerves of intracerebral origin . Axon terminals are found in close proximity to pericytes and sometimes the axons abut directly on endothelial cells (Rennels and Nelson, 1975 and Swanson et al.,1977).

These observations seem to have particular relevance in view of the finding that capillary walls contain immunohistochemically visible contractile protein (Owman et al., 1977)

which might suggest the possibility that the capillary endothelial cells and pericytes directly participate in the control of cerebral blood flow .

Human omental veins have noradrenaline nerve terminals, a short distance from the endothelium . Cholinergic and adrenergic unmyelinated axons and terminals occur in the intima of the pulmonary veins of the mouse (Hung and Loosli, 1977) .

Otherwise , innervation of blood vessels is generally restricted to the adventitia(arterial) or adventitia and media in veins .

Surface Receptors Of The Endothelium

A number of receptors for various hormones and neurotransmitters (α , B receptors agonists, acetylcholine , 5-hydroxytryptamine , angiotensin and histamine) have been shown to be present in cultured endothelial cells as determined by increased level of intracellular cyclic nucleotides, after exposure of the cultures to various vasoactive compounds (Buonassisi and Venter, 1976 and Schafer et al., 1980) With the exception of histamine and angiotensin II which increased only the level of intracellular cyclic AMP, all other hormones increased the level of both cyclic nucleotides .

Recent evidence indicates that pure endothelial cells cultures derived from dissociated cerebrovascular fraction contain adenyl cyclase activity which can be stimulated by catecholamines and prostaglandins and inhibited by adenosine angiotensin I and II, Gamma aminobutyric acid and vasoactive intestinal peptide while unaffected by acetylcholine, histamine, 5-hydroxytryptamine, glycine, glutamine, bradykinin, neurotensin and vasopressin (Karnushina et al., 1982) . The susceptibility of cerebrovascular endothelial cells adenyl cyclase system to these vasoactive substances together with the reported endothelial presence of contractile elements (Spatz and Mrsulja, 1982 support the proposal of the endothelial involvement in the regulation of cerebrovascular blood flow .