Non-specific gap junction action of connexin-mimetic peptides in the rat basilar artery

R.E. Haddock,[1,2] K.R. Meaney,[1] S.L. Sandow[2] and C.E. Hill,[1] [1]John Curtin School of Medical Research, Australian National University, Canberra, ACT 0200, Australia and [2]School of Medical Sciences, University of New South Wales, NSW 2052, Australia.

Homo- and heterocellular coupling of endothelial and smooth muscle cells *via* gap junctions is integral for arterial function. Attenuation of gap junction activity by putative gap junction blockers, such as the connexin (Cx)-mimetic peptides and the licorice derivatives, carbenoxolone and glycyrrhetinic acid, has been used as evidence implicating gap junctions in arterial function. The Cx-mimetic or Gap peptides, which mimic segments of the amino acid sequence of the extracellular loops of particular Cx proteins, are the current gap junction blockers of choice. The present study examined Cx-mimetic peptide (^{37,43}Gap27/^{40}Gap27) specificity in the juvenile male rat basilar artery. Animals were anaesthetized (44/8mg/kg^{-1} ketamine/xylazine, i.p.) and membrane potential, diameter and Ca^{2+} dynamics examined in intact and endothelium-denuded arteries. Vessel anatomy and Cx expression was examined using high resolution ultrastructural and confocal immunohistochemistry. Spontaneous vasomotion (rhythmical contractions) was observed. Separately, ^{37,43}Gap27 and ^{40}Gap27 abolished vasomotion and ^{40}Gap27 desynchronized adjacent smooth muscle cell $[Ca^{2+}]_i$ oscillations, in a similar manner to endothelial removal. However, peptides also reduced smooth muscle cell $[Ca^{2+}]_i$ oscillations and ^{37,43}Gap27 produced hyperpolarization. Control peptides also affected membrane potential and rhythmicity. Cx37 was present in the *tunica media*, whilst Cxs37, 40 and 43 were present between adjacent endothelial cells. Myoendothelial gap junctions comprised of Cxs37 and 40 connected endothelial and smooth muscle cell layers. Cx-mimetic peptide effects are not readily explained by specific action at gap junctions. Consistent with previous studies on specificity of the licorice derivatives, data suggest that, in addition to potential gap junctional effects, the Cx-mimetic peptides may act on ionic mechanisms unrelated to gap junction function; thus suggesting caution with their subsequent use in studies of gap junction function.