# Initiation and coordination of vasomotion in rat cerebral arteries

*R.E. Haddock, T.D. Brackenbury and C.E. Hill, Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia.*

In cerebral blood vessels, rhythmical contractions in vessel diameter, or vasomotion, play an important role in determining blood flow and vascular resistance. In mesenteric arteries, the endothelium and cGMP have been shown to be essential for initiating vasomotion, presumably through the release of nitric oxide (NO) (Peng *et al.*, 2001), while cell coupling is suggested to coordinate calcium oscillations (Sell *et al.*, 2002). Vasomotion in the basilar artery is dependent on intracellular calcium stores and an interplay between voltage activated calcium and potassium channels (Haddock & Hill, 2002), while the role of the endothelium and cell coupling is unknown. We have therefore investigated the role and distribution of vascular connexins (Cx) and the role of the endothelium in the initiation and coordination of vasomotion in the juvenile rat basilar artery.

Wistar rats (14-17 days) were anaesthetised with ether and decapitated (Animal Experimentation Ethics Committee, ANU). The basilar artery and its branches were isolated and pinned in a recording chamber which was perfused with physiological saline. Changes in membrane potential were measured with sharp microelectrodes (140-220 MΩ), which were filled with propidium iodide to identify the impaled cells. Changes in arterial wall or individual smooth muscle cell (SMC) intracellular calcium ($[Ca^{2+}]_i$) were assessed using Fura 2-AM, and either photometry or an intensified CCD camera respectively. Contractions of the same vessels were simultaneously recorded using videomicroscopy. Immunohistochemistry was performed on intact vessels following perfusion fixation (2% paraformaldehyde in phosphate buffer) and imaged using confocal microscopy. Serial section electron microscopy was used to investigate the presence of myoendothelial gap junctions (MEJGs).

Under control conditions, rhythmical depolarisations and $[Ca^{2+}]_i$ oscillations in both the arterial wall and in individual SMCs preceded rhythmical contractions. Membrane potential recordings from either SMCs or ECs were not significantly different and serial section electron microscopy confirmed that MEGJs connected the endothelium to the SMCs. The NOS inhibitor L-NAME (10µM) and the selective guanylate cyclase inhibitor ODQ (10µM) increased the frequency and amplitude of rhythmical activity and constricted the vessel. ODQ, but not L-NAME, hyperpolarised the vessel. Removal of the endothelium resulted in irregular contractions, asynchronous $[Ca^{2+}]_i$ oscillations in adjacent SMCs and a small depolarisation of the vessel. Addition of ODQ to endothelial denuded preparations prevented the constriction and augmentation of residual rhythmical activity induced by cGMP inhibition, but had no effect on the hyperpolarisation. Cx37, 40 and 43, but not Cx45, were found in the endothelium, while Cx 37, 43 and 45 were expressed to a lesser extent in SMCs. The gap junction uncoupler $^{37,43}$Gap 27 (100µM) abolished rhythmical activity and hyperpolarised the SMCs, while $^{40}$Gap 27 resulted in irregular contractions and asynchronous $[Ca^{2+}]_i$ oscillations in SMCs, but had no effect on membrane potential.

We conclude that the endothelium is essential for the coordination but not initiation of $[Ca^{2+}]_i$ oscillations and vasomotion in the basilar artery. This does not occur through the release of NO and activation of a depolarising current, but may instead be due to electrical coupling through MEGJs containing Cx40. The hyperpolarisation caused by ODQ suggests effects on ion channels of additional cGMP within SMCs. The persistence of vasomotion in the presence of ODQ confirms that cGMP was also not responsible for the initiation of vasomotion. Together the data indicate that the mechanism responsible for the initiation of vasomotion in cerebral arteries differs from that in systemic vessels.

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