Recruitment of the Vascular Endothelium into Neurovascular Coupling

Thomas Longden, Mark Nelson. University of Vermont, Burlington, VT, USA

Increased neuronal activity in the brain leads to elevated local blood flow to satisfy the increased oxygen and nutrient demand of the active tissue, a process known as neurovascular coupling (NVC). Although the vascular endothelium has a major role in the modulation of blood flow, it has not been thought to have an active role in NVC. However, NVC could conceivably engage the vascular endothelium through a variety of possible mechanisms, including changes in flow, mechanical forces, and activation by substances released from astrocytes and neurons. Here we explore the hypothesis that the endothelium has an important role in NVC.

Two-photon laser scanning microscopy with simultaneous bright field DIC imaging was used to investigate the effect of pharmacological interventions in a neurovascular coupling model evoked by neuronal depolarization *via* electrical field stimulation. Brain slices were prepared from 2-3 month old male C57 Bl/6 mice. Intraparenchymal arterioles were selected for study and vessels were pre-constricted by bathing the slices in U46619 (125 nM; 29±4% tone, n=15) to mimic vascular tone *in situ*. In some experiments, the endothelium was selectively loaded with the membrane potentiometric dye di-8-ANEPPS (20 µM) *via* transcardiac perfusion. Data are expressed as mean±s.e.m and were analysed using the paired t-test.

Blockade of neuronal nitric oxide synthase (NOS) with 7-NI (100 µM) reduced the magnitude of EFS-evoked vasodilatation by 63% (n=7) with no effect on basal vessel tone (n=5). Blockade of both neuronal and endothelial NOS isoforms with L-NNA (100 µM) caused a tonic constriction (17±4%, n=5) and reduced EFS-evoked vasodilatation by 89% (n=7). In the absence of U46619, L-NNA constricted arterioles by 28±0% and reduced neuronally-induced vasodilatation by 90% (n=5).

The 'sustained-phase' of the EFS-evoked vasodilatation was shortened by $K_{Ca}2.3$ and $K_{Ca}3.1$ channel blockade with a combination of apamin (100 nM) and TRAM-34 (1 µM), where vessel diameter was 27±11% more dilated than baseline 50 seconds after EFS under control conditions and just 2±4% more dilated 50 seconds after EFS after apamin and TRAM-34 treatment (n=9). Moreover, EFS-evoked arteriolar dilation was accompanied by endothelial hyperpolarization, as indicated by a 1.4±0.4% increase in the 620:560 nm fluorescence ratio of di-8-ANEPPS (n=7).

These data demonstrate that the vascular endothelium becomes hyperpolarized during neuronal activity-evoked vasodilatation, possibly by activation of endothelial cell $K_{Ca}2.3$ and $K_{Ca}3.1$ channels. $K_{Ca}2.3$ channels are also present in neurons, where block would be expected to increase neuronal excitability. $K_{Ca}3.1$ channels are also present in astrocytes, and may contribute to NVC[1]. The differential effects of inhibition of all NOS isoforms compared with neuronal NOS alone suggest that block of endothelial NOS affects NVC. Blocking endothelial NOS or $K_{Ca}3.1/K_{Ca}2.3$ significantly increases the tone of isolated pressurized arterioles[2], which could affect the degree of NVC[3]. Collectively, our data are consistent with the concept that the vascular endothelium is actively engaged during neurovascular coupling.

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