NO SUPPRESSES MYOGENIC TONE IN RAT MIDDLE CEREBRAL ARTERY BY ACTIVATING BK_{Ca} CHANNELS

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The rat middle cerebral artery exhibits a strong myogenic response. This myogenic response is normally suppressed by a basal release of NO as inhibition of nitric oxide synthase (NOS) causes both vasoconstriction and smooth muscle depolarization associated with oscillations in tension and membrane potential (vasomotion; McNeish *et al.,* 2005).We wished to characterise the cellular mechanisms which underpin the vasoconstrictor effect of inhibitors of NOS by simultaneously measuring smooth muscle cell (SMC) membrane potential and tension or SMC $[Ca^{2+}]$ and tension

Male Wistar rats (200-300g) were killed by cervical dislocation and exsanguination. The brain was removed and placed immediately in ice-cold Krebs solution. Segments of the middle cerebral artery (length, ~2mm; diameter, ~150 μm) were mounted in Krebs solution (or MOPS solution for imaging experiments) in a Mulveny-Halpern myograph. Smooth muscle cell membrane potential (E_m) was recorded with sharp glass microelectrodes (tip resistances of 80-120 MΩ) filled with 2M KCl. Changes in SMC $[Ca^{2+}]$ were recorded on a confocal microscope (Olympus FV-300) with vessels that had been pre-incubated with a Ca^{2+}-sensitive fluorescent dye (Fluo-4AM). Data are mean ± s.e.mean of 4 or more animals. Statistical comparisons were made using one-way ANOVA with Bonferroni's post-test.

Inhibition of NOS with L-NAME (100 μM) caused SMC depolarization associated with constriction and the development of oscillations in E_m and tension. L-NAME also caused an increase in SMC $[Ca^{2+}]$ and synchronisation of Ca^{2+} waves, that appeared to be temporally linked to changes in tension and E_m. The effect of L-NAME was mimicked by the guanylyl cyclase blocker, ODQ (10μM) and the BK_{Ca} blocker, iberiotoxin (100nM). Relaxation and hyperpolarization elicited by the NO donor DEA-NONOate (300 nM) was inhibited by iberiotoxin and ODQ but not by glibenclamide (10 μM), apamin (50 nM) or TRAM-34 (1 μM). L-NAME-induced constriction could be fully reversed by nifedipine (1μM, block of L-type Ca^{2+} channels), which also abolished oscillations in SMC $[Ca^{2+}]$. In the presence of L-NAME, niflumic acid (100 μM, blockade of calcium activated chloride channels; Cl_{Ca}) relaxed and hyperpolarised middle cerebral arteries but had little effect on SMC $[Ca^{2+}]$, a structurally distinct Cl_{Ca} blocker, DIDS (300 μM), failed to elicit relaxation or hyperpolarization.

In rat middle cerebral arteries the basal NO synthase activity results in suppression of myogenic tone. Inhibition of NOS causes vasoconstriction and depolarization involving the entry of Ca^{2+} through L-type Ca^2 channels. The vasomotion appears to be underpinned by oscillations in membrane potential and SMC $[Ca^{2+}]$. The peak of oscillations in E_m (circa -25mV) and the effect of a blocker of Cl_{Ca}, niflumic acid, suggest that a chloride conductance may be involved in the L-NAME induced constriction and vasomotion; however a structurally distinct blocker of Cl_{Ca}, DIDS, had no effect. Blockade of BK_{Ca} mimics the effects of L-NAME suggesting that NO normally suppresses myogenic tone by activation of SMC BK_{Ca} channels.

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