Location of voltage dependent calcium channel subtypes controls different aspects of cerebrovascular function

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Vasomotion, or rhythmical vasoconstriction, is a common feature of the cerebral vasculature *in vivo* and *in vitro* (Fuji *et al.,* 1990; Haddock & Hill, 2002). The mechanism underlying vasomotion in cerebral vessels relies on a tonic depolarisation following release of calcium from intracellular stores and the activation of a membrane oscillation involving the alternate opening and closing of L-type voltage dependent calcium channels (VDCCs) and calcium activated potassium channels (Haddock & Hill, 2002). Vascular tone of cerebral arteries has also been reported to depend on calcium influx through L-type VDCCs (Alborch *et al*., 1995). The aim of the present study was therefore to investigate how VDCCs could participate in these two different vascular functions. Basilar arteries taken from juvenile Wistar rats, which had been deeply anaesthetised with ether and exsanguinated, were used in electrophysiological, anatomical and molecular biological studies to determine the expression and functional role of VDCC subtypes in vasomotion and vascular tone. Studies in which the membrane potential of the basilar artery was altered with current injection were conducted on short isopotential vessel segments. Blockade of L-type channels with nifedipine abolished vasomotion but had no effect on vascular tone, although hyperpolarisation of short arterial segments did produce immediate relaxation. This relaxation was still seen in the presence of nifedipine. In contrast, depolarisation of quiescent and relaxed vascular segments evoked constriction and vasomotion in control Krebs' solution, while in the presence of nifedipine, vasoconstriction but not vasomotion was evoked. Real time PCR showed that L- and T-type VDCCs were strongly expressed in the main basilar artery and side branches, with $Ca_V3.1$ and $Ca_V1.2$ the predominant subtypes. Confocal microscopy confirmed that these two channels were strongly expressed as protein and additionally demonstrated their differential distribution in the cell membrane of the vascular smooth muscle cells. The T-type VDCC blockers, mibefradil, pimozide and flunarizine, relaxed and hyperpolarised basilar arteries, while low concentrations of nickel chloride had no effect. When the IP_3 pathway was blocked with the phospholipase-C inhibitor, U73122, to produce relaxation, the addition of Krebs' solution containing 40 mmol/L KCl and nifedipine evoked depolarisation and constriction and this was significantly reduced by mibefradil. The results suggest that vasomotion in the rat basilar artery depends on calcium influx through L-type VDCCs, while vascular tone results from calcium influx through nifedipine-insensitive VDCCs with pharmacology consistent with $Ca_V3.1$, T-type channels. The ability of the calcium traversing these different VDCCs to participate in different vasomotor responses suggests that these channels and their immediate surroundings are physically separated in microdomains within the cell membrane.

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