Novel nifedipine-insensitive high voltage activated calcium channels play a role in vascular tone of cerebral arteries

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Calcium channels are common therapeutic targets for the treatment of cardiovascular disorders such as hypertension (Ishikawa *et al.*, 1997), however they have not always been as successful as might be expected against vasospasm and stroke. Recently a novel high voltage activated, nifedipine-insensitive, mibefradil-sensitive calcium channel was described in small mesenteric arteries of guinea pigs and rats (Morita *et al.*, 1999, 2002), and called the "M-type" voltage dependent calcium channel (mVDCC). This channel has also been found in rabbit mesenteric arteries, where it is suggested to play a role in diameter regulation (Itonaga *et al.*, 2002). The aim of the present study was to determine if cerebral arteries possess similar nifedipine-insensitive VDCCs which could be used as targets for cerebrovascular disorders.

Juvenile (14-17 day old) male Wistar rats were anaesthetized with ether and decapitated. The basilar artery was removed from the brain and superfused with physiological Krebs solution at 33-37°C. Diameter was monitored as a measure of vascular tone using an edge-tracking computer program. Membrane potential was measured with sharp intracellular microelectrodes (100-180 MΩ), and current pulses (1-2 min) were applied to short segments of artery (less than 800µm) using discontinuous current clamp mode (Axoclamp 2B). Change in intracellular calcium concentration ($[Ca]_i$) was measured with the ratiometric calcium indicator Fura-2 AM and a photometry system. After 30 minutes the arteries developed spontaneous rhythmical oscillations in diameter (vasomotion) and membrane potential with the most negative potential around -45mV. Application of the L-type VDCC blocker, nifedipine, abolished vasomotion but did not alter tone, membrane potential or $[Ca]_i$ in basilar arteries, while inhibition of the IP_3 pathway with U73122 also abolished vasomotion but caused hyperpolarization, relaxation and a decrease in $[Ca]_i$. Small hyperpolarizing current steps which took the membrane potential to -50mV caused immediate abolition of vasomotion and relaxation. Relaxation occurred in the presence or absence of nifedipine. Application of the T- and M-type VDCC blocker, mibefradil, hyperpolarized and relaxed the artery, decreasing $[Ca]_i$, while the T-type VDCC blocker, nickel chloride, only relaxed the artery at a high non-specific concentration (1mM). After the artery was hyperpolarized and relaxed with U73122, application of 40mM KCl caused depolarization and constriction in the presence of nifedipine. A similar result was obtained when 2-APB, an IP_3 inhibitor was present together with nifedipine, suggesting that the effect of voltage was not on calcium release from intracellular stores. Taken together the results suggest that nifedipine-insensitive, mibefradil-sensitive VDCCs play a role in vascular tone in the rat basilar artery. These channels are activated at depolarized potentials and rapidly closed by small hyperpolarizations. They are thus unlikely to be T-type VDCCs, which are activated at more negative potentials and rapidly inactivated during prolonged depolarization. We suggest that these nifedipine-insensitive high voltage-activated calcium channels may provide a novel therapeutic target for cerebrovascular disorders.

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