Investigating involvement of store-operated channel in stretch-induced axonal injury

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Diffuse axonal injury (DAI) is a common and potentially devastating pathology caused by shearing force during rapidly acceleration or deceleration of the head as may occur in traffic accidents, falls, assaults and blast. It has long been considered that elevated neuronal intracellular calcium after injury plays a crucial role in the axonal degeneration and disconnection after DAI (Wolf *et al.*, 2001). However, the mechanism by which calcium accumulates in axons is still not fully understood. One possibility is that axonal injury will deplete the calcium in the endoplasmic reticulum (ER), and then activate the store-operated channels (SOCs), allowing calcium influx into the axoplasm (Staal *et al.*, 2010). Several constituent proteins of SOCs are expressed on neuronal cell bodies, such as STIM1, ORAI1 and TRPCs. But there is no evidence to show whether these proteins are also expressed along the axon, which might be involved in DAI.

To investigate the possible role of the SOCs in DAI, we first established an *in vitro* DAI model by axonal uniaxial stretch. Briefly, elastic silicon chambers with high optical transparency were used for primary cortical neuron culture isolated from newborn mice. Before seeding cells into chambers, a silicone barrier (2 mm width $\times$ 2 cm length) was inserted into the middle of the chamber to create a “gap area” which allows only axons to grow into this area. After 7-11 days *in vitro* culture, neurons and axons were loaded with 2.5 μM fluo-4 AM calcium indicator and 3 μM Cell Tracker Red (CTR), which were used as a ratio measurement in order to correct for volume changes (Kilinc *et al.*, 2009). Chambers were transferred into stretch machine (STREX Cell Strain Instrument) which was placed on the stage of a confocal microscope. A single stretch of 20% was applied to the chamber over a period of 1s (onset time 500ms). Secondly, potential components of SOCs, STIM1, ORAI1, TRPC1, TRPC3, TRPC4, TRPC6, were examined for expression on neuronal axons by immunocytochemistry.

After stretch injury, increased axonal intracellular calcium could be detected by the increased Fluo-4/CTR ratio. We also observed formation of axonal beads, associated with axonal damage and degeneration. Immunocytochemistry results showed that STIM1, ORAI1, TRPC1, TRPC3, TRPC4 and TRPC6 are expressed on axons.

Our results so far confirm that DAI is associated with increased intra-axonal calcium and demonstrate the expression of candidate proteins associated with SOCs in the axon. The functional involvement of SOCs in DAI is currently under further investigation.

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