Mitochondria-induced hyperpolarization in mouse locus coeruleus neurons is dependent on Ca^{2+} entry but not intracellular Ca^{2+} release

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Locus coeruleus (LC) neurons are known to play a fundamental role in brain function, impacting on many physiological processes such as regulation of sleep and vigilance, learning and memory, behavioural flexibility, and a range of other functions (for review see Sara, 2009). Mitochondria are intracellular organelles that appear to be involved in vast range of different pathways, including energy production, neuronal death, oxidative stress, neurodegenerative diseases and their role in buffering intracellular Ca^{2+} and resultant impact on Ca^{2+}-dependent pathways (Ishii, Hirose & Iino, 2006; Lehninger, Nelson & Cox, 2008). In rat LC neurons, it has been demonstrated that mitochondrial disruption caused an increase in intracellular Ca^{2+} and activation of Ca^{2+}-activated K^+ channels and resultant membrane hyperpolarization (Murai *et al.*, 1997). Here, we demonstrate that the hyperpolarization caused by mitochondrial disruption in mouse LC neurons is dependent on external Ca^{2+} entry and not dependent on increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). The methods used for euthanizing mice were approved by the Animal Care and Ethics Committee at the University of Newcastle. The brain was rapidly removed with a slice containing the LC then prepared, allowed to equilibrate and placed on the stage of an upright microscope in a bath perfused with artificial cerebrospinal fluid (ACSF) at 35°C (de Oliveira *et al.*, 2010). Recordings were made from LC neurons using patch electrodes in whole cell recording mode. Mitochondrial disruption with the protonophore CCCP (1 μM) caused hyperpolarization or outward current in current and voltage clamp modes, respectively. This outward current was likely to be dominantly generated by Ca^{2+} activated K^+ channels of the SK type, as the conductance was largely blocked by Apamin (1 μM). The outward conductance was dependent on external Ca^{2+} entry, as determined using Ca^{2+}-free (0.5 mM EGTA) ACSF and Co^{2+} ACSF (Co^{2+} substituted for Ca^{2+}) solutions. This conductance was not inhibited when an internal pipette solution containing a high concentration of the Ca^{2+} buffer EGTA (15 mM) was used, suggesting that $[Ca^{2+}]_c$ was not involved in its activation. Ca^{2+} imaging demonstrated that CCCP increased intracellular Ca^{2+} in both ACSF and the Ca^{2+}-free ACSF. The latter observation combined with the finding that the CCCP-generated outward conductance was not activated in Ca^{2+}-free ACSF confirmed that increases in cytosolic $[Ca^{2+}]_c$ *per se* did not activate the outward conductance. Taken together, these results demonstrate that hyperpolarization induced by mitochondrial disruption using the protonophore CCCP causes Ca^{2+} entry and resultant Ca^{2+}-activated K^+ conductance that is independent of intracellular Ca^{2+} release from stores, but is dependent on external Ca^{2+} entry. This suggests that activation of this conductance occurs in a microdomain.

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