Huntingtin-associated protein 1 (HAP1) regulates exocytosis, vesicle localization and interacts with multiple vesicle proteins

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Huntingtin-associated protein 1 (HAP-1) has a greater binding affinity for mutant huntingtin, a key player in Huntington disease, than normal huntingtin. Subcellular localisation and protein interaction data indicate that the HAP-1 may be important in vesicle trafficking and cell signalling. However, no physiological evidence exists to verify this possibility.

We measured exocytosis using carbon-fibre amperometry on chromaffin cells cultured from HAP-$1^{-/-}$ (KO), and HAP-$1^{+/+}$ (WT) mice. Levels of exocytosis in WT (102.2 ± 10.2 exocytotic events, n=29) cells are significantly greater than in KO cells (60.4 ± 7.1, n=35; $P<0.01$). The duration of transient fusion pore opening is prolonged in KO cells (3.0 ± 0.1 ms) compared to WT (2.3 ± 0.1 ms, $P<0.05$) cells indicating that HAP-1 may stabilize the formation of the fusion pore. The size of the RRP is smaller in KO cells (19 ± 5.3, n=7) compared to WT cells (54.4 ± 8.9, n=7, $P<0.01$) and this is due to the reduced number of vesicles docked with the plasma membrane in KO cells. Using a proteomics approach, novel interactions between HAP-1 and known trafficking-related proteins have also been discovered. Also, the amount of glutamate released from KO cortical brain slices was significantly smaller (30 ± 5 nM/mg protein, n=4 animals) compared to WT slices (60 ± 6, n=4 animals, $P<0.01$) and the localization of synaptic vesicles is significantly altered in KO neurons.

Our study reports a novel role of HAP-1 as a regulator of neurotransmitter release by influencing the rate of exocytosis, the dynamics of fusion pore opening, the size of the readily releasable pool (RRP) of vesicles released immediately upon stimulation and *via* binding to proteins involved in vesicle trafficking.