Huntingtin-Associated Protein 1 (HAP-1) is a novel regulator of exocytosis

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Huntington's Disease (HD) is a fatal neurodegenerative disorder, the genetic cause of which is a mutation in the gene encoding the protein Huntingtin (Htt). This mutation causes an expansion of an N-terminal CAG repeat sequence translating to a polyglutamine extension in the protein. Due to the ubiquitous expression of mutant Htt, the explanation for the selective neurodegeneration seen in HD may be the altered protein interactions of mutant Htt. Htt interacts with multiple proteins including Huntingtin-Associated Protein 1 (HAP-1) (Li *et al.,* 1995). HAP-1 may be an important factor in HD pathogenesis as mutant Htt has a greater binding affinity for HAP1 than Htt (Li *et al.,* 1995). Based on its subcellular localisation and protein interactions, HAP-1 is thought to play a role in vesicle trafficking and microtubule transport.

This study aims to determine whether HAP-1 has an influence on vesicle exocytosis. Carbon fiber amperometry was used to detect exocytosis from single chromaffin cells isolated using collagenase (Type A, Roche) from the adrenal glands of dead mice at P0. A carbon-fibre electrode at +800 mV was placed on the surface of a chromaffin cell which was stimulated with a high K^+ (70 mM) solution for 60 seconds. We measured current caused by the oxidation of released catecholamines and analysed the number of current spikes, representing single exocytotic events, occurring in this time. Chromaffin cells were cultured from *HAP-1*$^{-/-}$ (KO), *HAP-1*$^{+/-}$ (Het) and *HAP-1*$^{+/+}$ (WT) mice. We found a similar level of exocytosis in WT (102.9 ± 13.4 exocytotic events, $n = 25$) and Het (91.3 ± 10.9, $n = 21$) cells whereas exocytosis in KO cells was reduced (60.1 ± 6.9, $n = 36$) significantly compared to either WT ($p < 0.01$) or Het ($p < 0.05$) cells. Analysis of individual amperometric spike shapes gives an insight into the characteristics of vesicle fusion pore kinetics. We observed no significant differences in spike shape between genotypes apart from the duration of the "foot signal". This signal precedes a full amperometric spike and is an indicator of fusion pore opening. We found foot duration to be prolonged in KO cells (2.09 ± 0.22 ms) compared to WT (1.55 ± 0.12 ms, $p < 0.05$) and Het (1.45 ± 0.08 ms, $p < 0.05$). The size of the readily releasable pool (RRP) is also regulated by HAP-1. We exposed cells to a hyperosmotic solution for 10 seconds and observed the number of exocytotic events subsequently occurring as a measure of the number of pre-fused vesicles, representing the RRP. The number of events that occurred in KO cells (19 ± 5.3, $n = 7$) was less than in WT cells (68.8 ± 8.3, $n = 4$, $p < 0.01$) or Het (46 ± 2.9, $n = 8$, $p < 0.05$) cells. These findings illustrate that HAP-1 has a previously unknown role in regulating exocytosis. Underlying this are alterations in the RRP size and in the rate of fusion pore formation. If HAP-1 is found to similarly affect neurotransmission in the brain then the potential may exist for an involvement of HAP-1 regulating synaptic activity and neuronal communication and possibly in HD pathogenesis.

Li X, Li S, Sharp AH, Nucifora F, Schilling G, Lanahan A, Worley P, Snyder S, Ross C. (1995) *Nature* **378:**, 398-402.