Huntingtin-associated protein 1 (HAP1) regulates exocytosis via multiple mechanisms

K. Mackenzie, X.F. Zhou and D.J. Keating , Discipline of Human Physiology, Centre of Neuroscience, Flinders University, Sturt Rd, Adelaide, 5042, Australia.

Subcellular localisation and protein interaction data indicate that HAP-1 may be important in vesicle trafficking and microtubule transport. However, no physiological evidence exists to verify this possibility. Our study reports a novel role of HAP-1 as a regulator of exocytosis by influencing the rate of exocytosis, fusion pore dynamics and the size of the readily releasable pool (RRP) which consists of vesicles released immediately upon stimulation. Chromaffin cells from the adrenal gland were used for our exocytosis assays. Cells were cultured from adrenal glands taken from dead 8 week old mice using collagenase (Type A, Roche). Carbon-fibre amperometry was used to investigate exocytosis in single chromaffin cells. We applied +800 mV to a carbon fibre electrode placed on the surface of a single chromaffin cell. We simultaneously 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. Similar levels of exocytosis triggers by a 70mM K^+ solution wer found in WT (102.2 ± 10.2 exocytotic events, n= 29) and Het (90.8 ± 11.5, n=20) cells while exocytosis in KO cells was significantly reduced (60.4 ± 7.1, n=35) compared to WT (p<0.01) or Het (p<0.05) cells. The duration of the pre-spike "foot signal", an indicator of fusion pore opening, was found to be prolonged in KO cells (3.0 ± 0.1 ms) compared to WT (2.3 ± 0.1 ms, p<0.05) and Het (2.9 ± 0.1 ms, p<0.05) cells indicating that HAP-1 may function in stabilizing the formation of the fusion pore. The size of the RRP is also regulated by HAP-1 as the number of vesicles undergoing exocytosis following treatment with a hyperosmotic solution in KO cells (19 ± 5.3, n=7) is less than in WT (54.4 ± 8.9, n=7, p<0.01) or Het (46 ± 9.2, n=8, p<0.05) cells. Real-time PCR also indicates the downregulation of exocytosis-related genes in KO cells. Our findings implicate, for the first time, the involvement of HAP1 in the regulation of exocytosis at multiple levels including vesicle localisation, membrane fusion and gene transcription.