RCAN1 (Regulator of Calcineurin 1) is a novel regulator of secretory vesicle exocytosis and fusion pore kinetics

M.P. Zanin,[1] M. Pritchard[2] and D.J. Keating,[1,3] [1]Molecular and Cellular Neuroscience Group, Department of Human Physiology and Center for Neuroscience, Flinders University, Adelaide, SA 5042, Australia, [2]Centre for Functional Genomics and Human Disease, Monash Institute of Medical Research, Monash University, Clayton, VIC 3168, Australia and [3]Prince Henry's Institute of Medical Research, Clayton, VIC 3168, Australia.

Regulator of calcineurin 1 (*RCAN1*) is a gene located on chromosome 21 that is over expressed in the brains of Down syndrome and Alzheimer's disease patients, diseases in which synaptic activity is negatively affected. RCAN1 interacts with calcineurin to inhibit its activity (Fuentes *et al.*, 2000). Calcineurin is a protein phosphatase that regulates transcriptional activity via the NFAT pathway and also dephosphorylates a number of proteins, including several involved in exocytosis and endocytosis. We have used mice that transgenically overexpress *RCAN1* (*RCAN1^{ox}*) and mice in which *Rcan1* expression is ablated (*Rcan1$^{-/-}$*) to investigate the role of RCAN1 in exocytosis (Keating *et al.,* 2008). We studied exocytosis using carbon fibre amperometry on chromaffin cells, a neuroendocrine model of neuronal secretion. Chromaffin cells were obtained from the adrenal glands of dead, 6-8-week-old male mice. Catecholamine release from individual chromaffin cells was measured using carbon fibre amperometry, which involved placing a carbon-fibre electrode at +800 mV onto a chromaffin cell and recording a current trace for a period of 60 sec from the start of stimulation. Cells were depolarised using a bath solution containing 70 mM K^+, which consistently triggered exocytosis. Single exocytotic events were observed as spikes on the current trace and analysis of these spikes yielded information on the number of exocytotic events, speed of the events and the amount of catecholamines released per event. An increase in *RCAN1* expression resulted in fewer exocytotic events (*RCAN1^{ox}* 37.5 ± 5.4, control 58.9 ± 6.5, $p < 0.001$, $n = 23$) as did ablation of *Rcan1* (*Rcan1$^{-/-}$* 30.5 ± 2.9, control 60.8 ± 6.9, $p < 0.001$, $n = 21$). These data indicate that a careful balance exists between *RCAN1* expression and optimum levels of exocytosis. We also found that RCAN1 regulates fusion pore formation between the vesicle and plasma membranes. The speed of vesicle fusion was proportional to expression levels of RCAN1, evident as a decrease in catecholamine release with increasing speed of exocytosis (*Rcan1$^{-/-}$* 338.2 ± 34.1 pC, control 221.7 ± 22.1 pC, $p < 0.01$, $n = 16$, *RCAN1^{ox}* 171.6 ± 16.2 pC, control, 275.3 ± 25.6 pC, $p < 0.01$, $n = 16$). These effects were downstream of calcium entry, as determined by fluorescent imaging of cells loaded with the Ca^{2+} indicator dye Fluo-3 AM (5 μM). The ready releasable pool, representing the number of pre-fused vesicles, was also unaffected by RCAN1 expression, as determined by exposure to a hypertonic bath solution containing 500 mM sucrose for 10 sec and measuring the subsequent number of exocytotic events. To determine if these effects of RCAN1 were due to regulation of calcineurin activity, cells were chronically treated with the calcineurin inhibitors cyclosporin A and FK506 (1 μM each). Chronic inhibition of calcineurin in control cells resulted in more rapid fusion pore kinetics, evident as a 53.2% decrease in catecholamine release per vesicle ($p < 0.05$, $n = 10$), making secretion similar to that seen in *RCAN1^{ox}* cells. In contrast, chronic calcineurin inhibition had no effect on the speed of fusion pore formation in *RCAN1^{ox}* cells. Chronic calcineurin inhibition also had no effect on the number of exocytotic events in either *RCAN1^{ox}* or control cells. These data demonstrate a novel role for *RCAN1* as a regulator of exocytosis. We observed regulation at two stages; the number of vesicles undergoing exocytosis, regulated independently of calcineurin, and the speed of each exocytotic event, regulated by RCAN1-dependent control of calcineurin activity.

Fuentes J, Genesca L, Kingsbury T, Cunningham K, Perez-Riba M, Estivill X, & De La Luna S. (2000). *Human Molecular Genetics*, **9:** 1681-90.

Keating D, Dubach D, Zanin M, Yu Y, Martin K, Zhao Y, Chen C, Porta S, Arnones M, Mittaz L, Pritchard M. (2008) *Human Molecular Genetics*, **17:**, 1020-30.