Commentary: Ion Channels, Fusion Pores and Exocytosis

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The key function of adrenal chromaffin cells is to secrete catecholamines in response to stress. Under stress, chromaffin cells are stimulated by cholinergic synaptic inputs resulting in catecholamine release. This stimulus-secretion coupling is mediated by activation of Ca^{2+} signaling through ion channels followed by fusion pore formation and exocytosis, and subsequent endocytosis. Several sessions at this symposium highlighted advances in the understanding of the role of ion channels in exocytosis and endocytosis, the mechanics of fusion pore formation and the role of Ca^{2+} syntillas and gap junctional communication in modulating catecholamine release. As well, new technologies used to investigate

stimulus–secretion coupling in catecholamine release were presented.

The functions of different types of calcium channels in exocytosis and endocytosis, as well as other ion channels in chromaffin cell function were discussed in two sessions: “Voltage-gated calcium channels and cell function” organized by *Emilio Carbone* (Italy) and “Structure and function of channels” organized by *Jiu-Ping Ding* (China). Among the speakers discussing the role of calcium channels in exocytosis/endocytosis were *Luis Gandia* (Spain) who gave an update on the role L-type Ca^{2+} channels (LTCCs; Cav1) play in the control of Ca^{2+} -dependent endocytosis. Gandia’s group brought new evidence that endocytosis in bovine chromaffin cells is likely associated with prolonged Ca^{2+} entry during cell depolarization and is likely associated with slowly inactivating Ca^{2+} channels rather than due to a colocalization of LTCCs with the clathrin-mediated endocytotic machinery. Indeed, immunostaining of L-, P/Q, and N-type channels showed no colocalization with clathrin, and application of roscovitine (a blocker of N and P/Q type channel inactivation) restored endocytosis when LTCCs were blocked by nifedipine. Thus, “compensatory” and “excess” endocytosis is driven by the Ca^{2+} entry through slowly inactivating Ca^{2+} channels. More extensive discussions on the role of different calcium channel subtypes on endocytosis in chromaffin cells is presented in a review by *Luis Gandia* below. *Antonio Miguel G. de Diego* (Spain) reviewed the possibility that LTCCs could exert control on exocytosis induced by acute hypoxia in rat embryo chromaffin cells. According to this, LTCCs (besides T-type channels) are also likely to be involved in the control of catecholamine release at the embryonic stage, when chromaffin cells are particularly sensitive to O_2 homeostasis and control cardiovascular responses. *Emilio Carbone* gave an overview of the ion channels controlling the spontaneous pacemaking of

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cultured mouse chromaffin cells by showing how LTCCs/Cav1.3 channels are tightly coupled to BK potassium channels and then showed how 24 h incubations with high doses of multiwalled carbon nanotubes (MWCNTs) reduced the number of spontaneously active chromaffin cells. Active cells that survived toxic effects had higher firing frequency and unaltered voltage-gated Ca^{2+} , Na^{+} and K^{+} current amplitudes. Interestingly, MWCNTs decreased the amount of released vesicles (the RRP decreased by 50 %) while the quantity of Ca^{2+} charge was unaltered, suggesting an interaction of CNTs with functional elements of the secretory apparatus. *Lu-Yang Wang* (Canada) provided insights into how the arrangement of voltage-gated Ca^{2+} channels and synaptic vesicles at the giant synapse of calyx of Held switches from “microdomain” to “nanodomain” coupling during development. Interestingly, deletion of the filamentous protein Septin 5 imparts immature synapses with striking morphological and functional features, reminiscent of mature synapses. Thus, Septin 5 appears to be a core molecular substrate that differentiates distinct release modalities at the central synapse. Besides voltage-gated Ca^{2+} channels, *Ronghua ZhuGe* (USA) discussed the role of Ca^{2+} syntillas, in regulating catecholamine release. Ca^{2+} syntillas are Ca^{2+} microdomains originating from internal stores. In contrast to Ca^{2+} microdomains due to Ca^{2+} influx, Ca^{2+} syntillas act as original inhibitory signals for chromaffin cell catecholamine release. In the adrenal tissue, catecholamine secretion is also under a local control exerted by gap junction between chromaffin cells. By combining electrophysiological, pharmacological and biochemical approaches in anesthetized mice, *Michel G. Desarménien* (France) showed that adrenal medullary gap junctions effectively modulate release of catecholamines in vivo. During chronic stress, gap junction expression is increased and their contributions to intercellular communication between chromaffin cells are enhanced, resulting in modulation of catecholamine secretion. Additionally, *Nathalie C. Guérineau* (France) showed that gap junction-built route between chromaffin cells display functional remodeling with changes during postnatal development from prevalent gap junction communication to mature synaptic transmission. Moreover, during cold stress in rats, increased electrical coupling between cells was observed. A review by *Guérineau, Desarménien, Carabelli, and Carbone* (below) sums up the importance of nicotinic, gap junction, and voltage-gated calcium channels in mediating stimulus-secretion coupling and catecholamine release during normal chromaffin cell function and in response to stress.

Jiu-Ping Ding discussed how voltage-activated potassium (BK) channel associated with $\beta 2$ subunits play a critical role during action potential firing in rat chromaffin cells. He identified the cytoplasmic binding site between mSlo1 α and h $\beta 2$ accessory subunits in BK channels as being important for physiological function. *Feng Qin* (China) presented studies

on temperature-gated ion channels which are biological thermometers with functions in thermal sensation and pain perception. *Xu-Hui Zeng* (China) reported studies showing that the Slo3 channel is the primary channel mediating the pH-dependent K^{+} current in spermatozoa required for normal sperm function. *Claire Zhang* (China) showed that small glutamate vesicles and lysosomes undergo exocytosis from a single cultured hippocampal astrocyte when triggered by Ca^{2+} .

During an exocytosis event of single vesicle release, the fusion pore is the initial fusion site between plasma membrane and the secretory vesicle. Many proteins are involved in this process, including SNAREs (VAMP, SNAP25, and syntaxin), synaptotagmins, and complexins. *Manfred Lindau* (USA) organized a session focusing on fusion pores and reported that fusion pore formation is activated by the synaptobrevin C terminus. *Seong An* (USA) discussed real-time visualization of complexin, a cytosolic protein that binds assembled SNARE complexes during single exocytic events in live cells. Their studies in PC12 cells revealed that complexin is recruited at a late step in exocytosis and modulates fusion pores composed of SNARE complexes. *Meyer B. Jackson* (USA) reported that synaptobrevin's (VAMP) membrane anchor complements syntaxin's to control fusion pore flux. Initial proteinaceous pore formation is followed by expansion to a lipid pore and this requires induction of high membrane curvature. *Ralf Mohrmann* (Germany) discussed the formation of SNARE complexes between vesicle and target membranes as a central step in membrane fusion. He showed in living cells that fast vesicle fusion which is typically found in synapses of neurosecretory cells requires at least three functional SNARE complexes, while slower release might occur with fewer complexes. *Ralf Mohrmann*, together with *Jacob Sorenson*, have reviewed thoroughly in a chapter below the role of SNARE complexes in vesicle priming, docking, fusion pore formation and fusion rates, steps required for vesicle exocytosis.

The role of lipids in exocytosis was also discussed at this meeting in a session organized by *Jens Coorssen* (Australia) who presented his studies on the involvement of cholesterol, poly-phosphoinositides and phosphatidic acid in vesicle priming, docking, and fusion. The importance of lipids and lipid rafts, microdomains rich in cholesterol and sphingolipids, in ion channel and fusion pore function was discussed by *Amy Tse* and *Frederick Tse* (Canada). Their studies showed that arachidonic acid (AA) is generated upon agonist stimulation and depending on the ion channels expressed and the cell type, action of AA on cellular excitability by acting via highly calcium permeable channels, can be stimulatory or inhibitory. Their recent findings also showed that breakdown of sphingomyelin from lipid rafts to liberate cholesterol can transiently stabilize the fusion pore during exocytosis. Together, they have elaborated on the role of lipids in fusion pore and ion channel function in a

review below. *Robert Zorec* (Slovenia) discussed interesting findings that the fusion pore properties differ during spontaneous and stimulated exocytosis using anterior pituitary cells as a model system. He suggested that the fusion pore may be an energetically stable structure at rest, exhibiting subnanometer diameter, too small for exit of signaling molecules, but enlarges during stimulation. These changes in properties with stimulation appear to be regulated by proteins and lipids. *Marko Kreft* (Slovenia) showed that small lipidic (sphingosine-like) molecules affect atrial natriuretic peptide vesicle trafficking in astrocytes.

Two sessions: “New imaging methods for cell signals” and “Physiological recordings in chromaffin cells” organized by *Liang-Yi Chen* (China) and *Nathalie C. Guérineau*, respectively, were dedicated to innovative new technologies used to investigate stimulus–secretion coupling, both in iso-

lated chromaffin cells and in the adrenal medullary tissue in vivo. These included talks by various experts on use of total internal reflection fluorescence microscopy (*Ron Holz*, USA), PALM (*Annya Smyth*, UK), and planar nanocrystalline diamond microelectrode arrays, an elegant technique allowing simultaneous amperometric detection of catecholamine secretion in multiple chromaffin cells (*Valentina Carabelli*, Italy). Along the same lines, *Chien-Yuan Pan* (Taiwan) used nanowire field-effect transistor and atomic force microscopy to monitor catecholamine exocytosis at a very high temporal/spatial resolution. Additionally, recent advances in the ability to simultaneously combine splanchnic nerve stimulation and electrophysiological recording of cell excitability with plasma catecholamine assays in anesthetized rodents allows one to convincingly analyze the in vivo regulation of adrenal excitation–secretion coupling.