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Recent advances in chromaffin cell biology: Summing up the last International Symposium on Chromaffin Cell Biology

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ABSTRACT

The International Symposium on Chromaffin Cell Biology (ISCCB) brings together a group of approximately 150 scientists from around the world who meet every 2 years to discuss recent advances in our understanding of biogenesis and motion of secretory vesicles, synthesis, storage and release of secreted products (catecholamines, chromogranins, ATP), and mechanisms involving the excitation-secretion coupling, membrane ion channels, intracellular calcium homeostasis and exocytosis. The development of new technologies that allow an accurate measurement of catecholamines, vesicle motion, exocytosis, etc. are also analyzed. The 12th ISCCB, organized by Ricardo Borges, took place on September 20-26, 2003, in La Palma, Canary Islands, Spain. In this article we describe the most recent and significant contributions to the 12th ISCCB.

Key terms: chromaffin cells, calcium channels, nicotine receptors, exocytosis, chromaffin granules, chromogranins.

Chromaffin cells from adrenal medulla are neuroendocrine cells that, like sympathetic neurons, derive from neural crest precursors and release catecholamines and other substances, such as ATP and chromogranins, into the bloodstream in response to stressful situations. These cells have been employed for many years as an experimental model to search for the mechanisms involved in the exocytotic process, biogenesis of secretory vesicle, and cell differentiation, among other issues. In the 12th ISCCB, we discussed the new understanding of the mechanisms involved in biogenesis and motion of chromaffin vesicles and exocytosis, regulation of calcium channels and nicotine receptors, and the function of chromogranins and their derived peptide. We also analyzed the development of new technologies for the measurement of catecholamines, vesicle motion, exocytosis, gene expression and mitochondrial pH.

Calcium channels and nicotine receptors

As neurons, human, mouse, and bovine chromaffin cells in primary cultures express L, N and P/Q subtypes of Ca^{2+} channels (Gandía et al., 1995; López et al., 1994; Gandía et al., 1998; García-Palomero et al., 2000). In the latest symposium, Aaron Fox (University of Chicago), Emilio Carbone (University of Torino), and Elizabeth Seward (University of Sheffield, UK) showed how distinct intracellular molecules modulate these channels. Thus, Aaron Fox and co-workers proposed that the auxiliary beta-2-a subunit regulates N-type channel inactivation. The beta-2-a subunit regulatory effect depends on its palmitoylation. In adrenal chromaffin cells, N-type channel inactivation is also regulated by calmodulin, as proposed by Robert Wykes and Elizabeth Seward.

On the other hand, the second messenger AMP cycle increases L-type calcium

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current, as demonstrated by Emilio Carbone and his co-workers. Also, the Carbone group demonstrated that AMP cycle up-regulates T-type channels.

Nicotine receptors are also regulated by different molecules. As demonstrated by Antonio García (Universidad Autónoma de Madrid) and his collaborators, choline, a degradation product of acetil-choline, activates alpha-7 receptor and blocks the alpha-3-beta-4 receptor. It has been proposed that $\alpha_3\beta_4$ and α_7 are the two primary nicotine receptor subtypes expressed on bovine chromaffin cells (Criado et al., 1997; Campos-Caro et al., 1997; López et al., 1998; Herrero et al., 2002), but in this symposium, Bruce Livett's (University of Melbourne) and Antonio García's groups suggest the presence of a heteromeric complex receptor formed by alpha-3, alpha-7 and beta-4 subunits. Interestingly, chronic nicotine up-regulates the alpha-3 beta-2 receptor, as demonstrated by Andrea Nistri (International School for Advanced Studies, Triste, Italy). Chronic treatment of chromaffin cells with nicotine also increases the levels of the anti-apoptotic proteins Bcl-Xl and Bcl-2 and the calcium binding protein, calbindin-D-28-k, as demonstrated by Manuela López group from Madrid.

Exocytosis

The release of neurotransmitters and hormones occurs when vesicles fuse with the plasma membrane in a calcium-dependent manner. The three SNARE (soluble NSF attachment protein receptor) proteins, VAMP (Vesicle Associated Membrane Protein), SNAP-25 (Synaptosomal Associated Protein of 25 kDa) and syntaxin are implicated in the final step of vesicular exocytosis, which is the fusion of membranes (Schiavo et al., 2000). Furthermore, the vesicle-associated Ca^{2+} binding protein, synaptotagmin, is required for the coupling between intracellular Ca^{2+} rise and secretory vesicle fusion (Koh and Bellen, 2003). These proteins are also involved in exocytosis of chromaffin cells (Burgoyne and Morgan, 2003).

During the present symposium, we discussed the recent understandings of the molecular components of the exocytosis, such as the calcium-independent binding of synaptotagmin to SNAREs (Colin Rickman from Cambridge, UK), the involvement of annexin 2 in the formation of lipid rafts during exocytosis (Marie-France Bader, CNRS, Strasbourg), and the contributions of SNAP-23 and SNAP-25 isoforms to driving secretion (Michel Wilson, Erwin Neher and colleagues).

The interaction of Sec-1 with SNARE (David Apps, University of Edinburgh), the syntaxin–independent role of Munc18 in exocytosis (Robert Burgoyne, University of Liverpool) and the binding of the cytosolic proteins complexins 1 and 2 to the SNARE complex (Christian Rosenmund, Max-Planck Institutes) were also discussed.

Biogenesis and motion of chromaffin granules

In relation to granule biogenesis, Y. Peng Loh (NIH) reported that chromogranin A regulates the expression of secretory granule proteins at the post-translational level, probably controlling the protein stability at the Golgi. Y. Peng Loh and colleagues also proposed that Chromogranin A regulates the transcriptional level of aquaporin-1, a water-channel protein, purified from secretory granules.

Regarding vesicle dynamic, David Apps, Robert Chow and their co-workers have designed a chimera between the vesicle protein atrial natriuretic factor and the fluorescent timer protein ds-Red-E-5, a protein that progressively shifts its fluoresce emission from green to yellow and finally to red. They suggested that secretory granules are spatially and functionally segregated according to age and that the newer granules are preferentially recruited for exocytosis (Duncan et al., 2003).

Concerning the vesicle motion through the cytoskeleton, Francois Darchen and collaborators (CNRS, Paris) reported that the GTPase Rab-27 and its effector MyRIP associate with chromaffin granules and

control their motion through the actin cortex. Myosin V also can associate to the secretory granules, as was demonstrated by José María Trifaro and his colleagues. They suggested that myosin V is a molecular motor protein involved in secretion.

Chromogranins and derived peptides

The acidic soluble proteins chromogranins A and B are co-secreted with catecholamines from the chromaffin granules (Laslop and Mahata, 2002). Significant evidence indicates that chromogranins and their derived peptides have several biological roles, such as the antimicrobial actions exhibit by some chromogranin-derived peptides, as reported by Mary Helen Metz-Boutigue (CNRS, Strasbourg). Yasothornsrikul and collaborators (University of California) reported that chromogranin A is an enhancer of the plasminogen activator. Angelo Corti (San Raffaele H Scientific Institutes, Milan) and colleagues reported that chromogranin A derived peptides vasostatins exhibit cardiosuppresive actions. The cationic domain of vasostatin 1 can regulate the smooth muscle functions, as demonstrated by Karen Helle (University of Berge) and co-workers. Parastatin, another chromogranin A derived peptide, exhibit autocrine inhibitory action of parathyroid secretion, as proposed by Brigitte Fasciotto Dunn and David Cohn from the University of Louisville. Furthermore, Mary Helen Metz-Boutigue reported that Chromogranin B synthesis and processing show nocturnal variation.

Chromogranin A has also been identified in chromaffin cells from the Zuckerkandl's organ, an extra-adrenal paraganglion located adjacent to the lower abdominal aorta (Emilio Fernandez-Espejo et al.). Intrabrain transplantation of those extra-adrenal chromaffin cells induces a gradual improvement of functional deficits in Parkinsonian rats, probably by the chronic release of glial cell line-derived neurotrophic factor and transforming growth factor-1 (Espejo et al., 2001).

Advanced technologies

Chromaffin cell has been a useful model for developing new techniques that explore biogenesis, motion and fusion of secretory granules, free cytosolic levels and quantal release of neurotransmitters and calcium contribution to secretion. The development of carbon fiber amperometry and membrane capacitance techniques to measure exocytosis are good examples of the advantageous utilization of chromaffin cells. Therefore, in this symposium, we especially emphasize new technologies.

Kevin Gillis and collaborators (University of Missouri) fabricated gold electrochemical electrode arrays in picoliter-sized wells for measuring catecholamine release from individual cells with millisecond resolution. Using this microchip technology, the measured amperometric spikes corresponding to quantal exocytosis (Chen et al., 2003).

Ira Milosevic, Erwin Neher and co-workers from the Max-Planck Institute adapted a membrane sheet technique to bovine chromaffin cells to measure exocytosis of individual secretory vesicles. Cells were subjected to a brief ultrasonic pulse to form a thin flat inside-out plasma membrane sheet with attached secretory and cytoskeleton elements.

Manfred Lindau and collaborators have established intracellular patch electrochemistry (for the direct measurements of cytosolic oxidizable molecules in single cells). They combined patch-amperometric recordings in the whole-cell configuration with amperometric or cyclic voltametric detection of cytosolic catecholamines (Mosharov et al., 2003).

Among other issues, we also analyzed the replacement of the patch clamp pipette with planar glass chip devices (Jan Behrends), the use of a green fluorescence protein mutant for monitoring mitochondrial pH (Tulio Pozzan) and bioluminescence imaging for dynamic measurements of sub-cellular calcium and hormone gene expression (Villalobos et al., 2002).

Finally, the design of the computational programs is worthy of note. Alan Schneider and collaborators have developed a

computational model of Ca^{2+} signaling in chromaffin cells, which quantitatively simulates the spatial-temporal patterns of Ca^{2+} signals, while Ricardo Borges and coworkers described a computational program for the analysis of individual secretory events measured by amperometry.

Future research directions

Several questions related to the molecular mechanism involved in exocytosis and vesicle motion remain still unanswered. For example, since annexin 2 promotes the formation (or stabilization) of lipids microdomains required for exocytosis (as proposed by MF Bader), do annexins determine the occurrence of kiss-and-run –or full fusion– events? Probably the use of the mentioned new technologies, such as the membrane sheet technique or microchip technology for measuring amperometric spikes will allow us to respond to those kinds of questions. In this regard, chromaffin cells will be the most adequate cellular model. We therefore hope to discuss the answers to these and other issues in the next International Symposium on Chromaffin Cell Biology, to be held in Chile in January 2006.

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