and quantitatively different in vesicle-rich and vesicle-poor synapses and depends on the spatial localization of the synapse and their number of neighbors, respectively. This variation could be the basis for specific information-processing circuits in the hippocampus.

3554-Pos
Simultaneous Optical-Electrical Measurement of the Delay between formation of the Fusion Pore and Proton Equilibration in Exocytosis of Single Vesicles
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Optical detection of neutralization of pH in granules or vesicles is often used to define exocytotic events. However, combined measurements of ensemble capacitance and pH-dependent vesicular fluorescence changes have suggested that the movement of protons only becomes possible after fusion pore expansion with a mean delay of > 300 ms (1). To enhance the temporal resolution of such measurements, we have combined capacitance recordings of single vesicle fusion in RBL cells transfected with synaptophysinfluor as a reporter of vesicular pH. To monitor cell capacitance steps due to exocytosis of single granules in whole cell patch-clamp mode, we used the piecewise linear technique. Internal solution contained 10 μM free Ca2+ and 300 μM GTPγS. Before establishing whole cell recordings, punctate fluorescence signals could be detected with excitation at 460 nm, while during perfusion with internal solution and excitation at 480 nm, punctate fluorescence signals gradually appeared at corresponding sites. Fluorescence increases clearly lagged capacitance steps by several 100 ms-seconds, supporting the idea that pH equilibration through the fusion pore is delayed. (1) Barg et al.: Neuron, 33, 287-299, 2002.

Intracellular Channels

3555-Pos
Functional Properties of SR Ca2+ and K+ Channels during Postnatal Development of Cardiac Muscle
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In adult (AD) heart, the sarcoplasmic reticulum (SR) contains Ca2+ and K+ channels presumably involved in controlling RyR-mediated SR Ca2+ release. These channels provide a countercurrent mechanism that attenuates the drop in Ca2+ driving force across the SR membrane, thereby preventing early termination of Ca2+ release. We showed that in newborn (NB), Ca2+ sparks occur with similar frequency than in AD but have shorter duration and smaller amplitude, implying an early termination of Ca2+ release. Although the functional properties of SR Ca2+ and K+ channels are thoroughly described in AD, little is known about their presence and their role in NB. Consequently, we first tested the hypothesis that the early termination of Ca2+ release in NB coincides with absence/low density of SR Ca2+ and K+ channels at this stage. To this end, the heavy microsomal fraction was obtained from 5-days-old NB and AD rat hearts and SR Ca2+ and K+ channels were reconstituted into artificial planar lipid bilayers. Our results indicate that Ca2+ and K+ channels can be reconstituted from NB heavy SR microsomes with a similar success rate (number of SR channel incorporations / total number of bilayers) than in AD (+0.2 for Ca2+ channels & -0.1 for K+ channels). Thus, an alternative mechanism would imply that in NB, smaller counterion fluxes result from different functional properties of SR Ca2+ and K+ channels. This assumption was tested by measuring their unitary conductance, open probability, and voltage dependence. The results in NB channels revealed no significant differences in any of these parameters in comparison to AD. Thus, we concluded that SR Ca2+ and K+ channels do not contribute to the developmental changes of Ca2+ release in NB cardiomyocytes. Supported by AHA-0655656Z to RMA.

3556-Pos
Role of TRIC-A Channel in Circulatory Function
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TRIC (trimeric intracellular cation) channels in the sarcoplasmic reticulum likely act as counter-ion channels that conduct monovalent cations in a synchronized manner with release of stored Ca2+. TRIC channel subtypes display differential expression patterns as TRIC-A is predominantly expressed in excitable tissues, including brain and muscle, and TRIC-B is present throughout many tissues. TRIC-A knockout mice are viable and fertile, while TRIC-B knockout mice exhibit neonatal lethality due to respiratory failure (Yamazaki et al., Development 2009), and double-knockout mice lacking both subtypes show embryonic cardiac failure (Yazawa et al., Nature, 2007). To resolve the physiological role of TRIC-A, we are currently focusing on abnormal circulatory function in TRIC-A-knockout mice during young adulthood. These mutant mice showed significant hypertension and bradycardia. Autonomic blocking agents (co-application of atropine and metoprolol) greatly improved the bradycardic condition without affecting hypertension in the mutant mice. This observation suggests that a hyperactive baroreceptor reflex leads to development of the bradycardic condition in the mutant mice. Blockers for vasoactive humoral factors, such as angiotensin, endothelin and vasopressin, did not significantly improve hypertension in the mutant mice, suggesting normal blood-vasopressor levels. Importantly, isometric tension measurements indicated that contractility is markedly impaired in aortic ring preparations from the mutant mice, and that acetylcholine-induced relaxation is hypersensitive in mutant mesenteric artery. Our results suggest a vital role for TRIC-A channels in the physiological regulation of vessel tonus by vascular smooth muscle and endothelial cells. To further examine the pathogenesis of hypertension at the molecular level, we plan to examine TRIC-A expression and agonist-evoked Ca2+ transients in smooth muscle and endothelial cells from TRIC-A-knockout and wild-type mice.