



Application Note
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Organization of β -adrenoceptor signaling compartments by sympathetic innervation of cardiac myocytes

The sympathetic nervous system regulates cardiac function through the activation of adrenergic receptors (ARs). β_1 and β_2 ARs are the primary sympathetic receptors in the heart and play different roles in regulating cardiac contractile function and remodeling in response to injury. In this study Professor Kobilka and colleagues from the Department of Molecular and Cellular Physiology at Stanford University, examined the targeting and trafficking of β_1 and β_2 ARs at cardiac sympathetic synapses *in vitro*.

This report details the first analysis of the organization of signaling molecules at the site of innervation of cardiac myocytes by sympathetic neurons. The team demonstrated that sympathetic ganglion neurons (SGNs) regulate the contraction rate of cultured myocytes and provide evidence that sympathetic innervation influences the structure of the myocyte membrane and the organization and distribution of β_1 and β_2 AR signaling compartments.

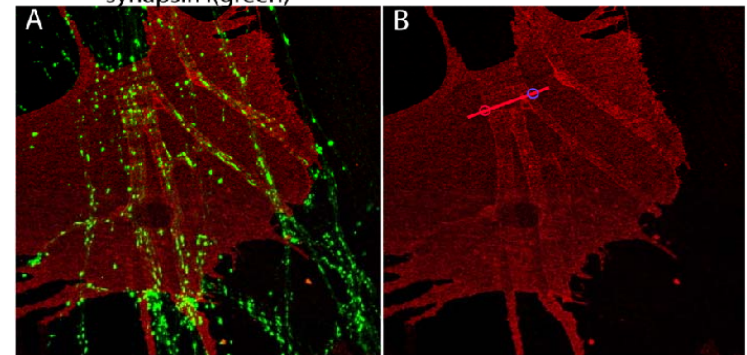
In order to visualize the β_1 and β_2 ARs in cardiac myocytes the team used expressed FLAG-tagged β_2 AR or HA-tagged β_1 AR via recombinant adenovirus in cocultures.

It was confirmed that the β_2 ARs are locally internalized at the sites of contacts, and this redistribution is regulated by neuronal activity. 3D rendered images were generated using **Volocity Visualization** and the wizard tool of the **Volocity** was used to select regions of interest.

It was found that the pattern of β_1 AR distribution is strikingly different from that of the β_2 AR. β_1 ARs are localized to zones of myocyte-SGN contact. The immunostaining pattern for HA-tagged β_1 ARs mirrors the shape of overlying axons, and the maximum accumulation of the receptors often surrounds the sites of synapsin I accumulation, which are presumed to be the sites of highest neurotransmission activity. **Volocity** was used to 3D render two-photon z sections, which permitted the observation of the invagination of the myocyte membrane at the point of contact with an SGN. This is shown in figure E and also as a QuickTime movie made with **Volocity**. This is available at (<http://www.jcb.org/cgi/content/full/jcb.200604167/DC1>).

This research indicates that β_1 ARs are stable residents at sympathetic synapses, whereas the β_2 ARs undergo dynamic trafficking events regulated by neuronal activity. A better understanding of the subtype-specific signaling of β_1 and β_2 ARs in cardiac myocytes in response to sympathetic nervous system activation could have implications for the prevention and treatment of heart failure.

HA-tagged β_1 AR(red)
synapsin I(green)



HA-tagged β_1 AR

β_1 ARs accumulate at sites of contact between SGNs and cardiac myocytes.

(A and B) Cardiac myocytes were cocultured with SGNs for 6 d, infected with recombinant adenovirus expressing HA-tagged β_1 AR, and cultured for an additional 24 h before immunostaining for HA (red) and synapsin I (green).

(E - G) X-Z view of the of the site of contact between a cardiac myocyte expressing HA-tagged β_1 AR and an SGN immunostained for HA (red) and synapsin I (green).

