**Animation Legend:** Instructions for viewing supplemental animation of contributions of IRP and HCSP pool of secretory granules to depolarization-dependent, Ca\(^{2+}\)-entry evoked quantal release and the HCSP to depolarization-independent, Ca\(^{2+}\)-release-dependent quantal release.

After opening the Powerpoint presentation in Powerpoint 2003, view the slide show (View \(\rightarrow\) Slide Show [F5]). The upper panel shows secretory surface of endocrine cells including: plasma membrane voltage dependent Ca\(^{2+}\) channels and Ca\(^{2+}\) extrusion pumps; granules of the low Ca\(^{2+}\) sensitivity IRP located near Ca\(^{2+}\) channels; granules of the high Ca\(^{2+}\) HCSP farther from the Ca\(^{2+}\) channels (both docked near the plasma membrane); and the more interior segment of endoplasmic reticulum containing Ca\(^{2+}\) sequestration pumps. Lower panel will later demonstrate the profile of cytosolic Ca\(^{2+}\), \([\text{Ca}^{2+}]_i\), as a function of distance along the membrane after stimulus provocation.

Click of down arrow of key pad or left side of mouse pad begins the sequences on depolarization-dependent, Ca\(^{2+}\)-entry evoked quantal release. First, a short action potential (AP), overshooting 0 mV, is evoked (see insert to upper panel). Ca\(^{2+}\) entry via voltage dependent Ca\(^{2+}\) channels provokes a highly localized rise in cytosolic [Ca\(^{2+}\)] in their vicinity but does not extend to a region of plasma membrane at which secretory granules are docked and hence fails to evoke exocytosis.

Second, on subsequent double click, a longer AP is evoked. The resultant, more prolonged Ca\(^{2+}\) entry increases the width of the [Ca\(^{2+}\)] profile so that it now extends into the vicinity of granules of the IRP and exceeds the [Ca\(^{2+}\)] threshold of granules of the IRP, thereby evoking exocytosis that is nearly synchronous with the AP.

Third, on a subsequent double click, a prolonged train of APs is evoked. Ca\(^{2+}\) entry with each AP provokes synchronous exocytosis from the IRP while the slow buildup of Ca\(^{2+}\) saturating buffers (expanding Ca\(^{2+}\) symbol in cytoplasm) further increases the width of the [Ca\(^{2+}\)] profile so that it now extends into the region of granules of the HCSP, exceeds the threshold for release from that pool, thereby evoking a slower asynchronous component of exocytosis.

Fourth, subsequent double click results in a plateau depolarization to roughly -20 mV lasting at least several seconds. Though the intensity of instantaneous local Ca\(^{2+}\) entry is not sufficient to exceed the threshold for the IRP, prolonged Ca\(^{2+}\) entry provides Ca\(^{2+}\) spillover into the vicinity of granules of HCSP to evoke exocytosis from this pool. With further single click, [Ca\(^{2+}\)] profile is then shown to wane as [Ca\(^{2+}\)] is sequestered by ER Ca pumps and extruded by plasma membrane.

Lastly, a subsequent click begins the sequence on depolarization-independent, Ca\(^{2+}\)-release-dependent quantal release. Note that the plasma membrane now contains an agonist (A) stimulated G-protein coupled receptor (R) while the ER membrane now contains a receptor-operated Ca\(^{2+}\)-release channel. Single click now reveals binding of A to R generates an intracellular second messenger (triangle) which activates the ER receptor-operated Ca\(^{2+}\)-release channel. Release of stored Ca\(^{2+}\) raises [Ca\(^{2+}\)] profile (again single click) to a level where granules of the HCSP are exocytosed. With the final click, the [Ca\(^{2+}\)] profile returns to background levels as [Ca\(^{2+}\)] is sequestered by ER Ca\(^{2+}\) pumps and plasma membrane Ca\(^{2+}\) extrusion pumps.