Ca\(^{2+}\)-dependent inactivation of L-type Ca\(^{2+}\) current (I\(_{\text{Ca,L}}\)) is an important mechanism for limiting Ca\(^{2+}\) entry and Ca\(^{2+}\) overload in cardiomyocytes. Moderate diastolic depolarization and increasing frequency of stimulation are however reported to augment Ca\(^{2+}\) entry through a Ca\(^{2+}\)-dependent mechanism. This phenomenon is referred to as facilitation of I\(_{\text{Ca,L}}\) and sarcoplasmic reticulum (SR) released Ca\(^{2+}\) may be important for this response. By employing a cardiac-specific Serca2 knock-out (KO) mouse with blunted SR function we have investigated the importance of SR function with regard to I\(_{\text{Ca,L}}\) facilitation. Serca2 flox/flox (FF) mice served as controls. Experiments were performed on enzymatically isolated cardiomyocytes from the left ventricle (22ºC). Application of 10 mM caffeine on Fluo-4 loaded cardiomyocytes elicited Ca\(^{2+}\) release in only 42% of KO cells vs. 100% in FF, and the magnitude of the caffeine elicited Ca\(^{2+}\) transients from KO cells with response was reduced by 82% compared to FF. Measurements of Ca\(^{2+}\) transients (1 Hz) in the presence and absence of caffeine showed that SR Ca\(^{2+}\) contributed to 86±3% of the Ca\(^{2+}\) transient in FF and only 7±11% in KO. The whole-cell patch clamp technique was used to record membrane currents. Moderate diastolic depolarizations induced depressed facilitation of I\(_{\text{Ca,L}}\) in KO cells compared to FF. Compared to I\(_{\text{Ca,L}}\) elicited from a diastolic holding potential of -80 mV, the greatest degree of facilitation was observed -40 mV. The integrated I\(_{\text{Ca,L}}\) was then increased by 10±4% in KO vs. 72±11% FF (P<0.05). Increasing the frequency of stimulation from 0.1 to 1 Hz induced a 68±15% (P<0.05) increase in integrated I\(_{\text{Ca,L}}\) in contrast to no significant change in KO. Thus, Serca2 KO induces a blunted SR function which is associated with a severe depression of frequency-, and voltage-dependent facilitation of I\(_{\text{Ca,L}}\).