Experiments were conducted on acute cerebellar slices of young (11-16 days old) rats. The calcium-sensitive fluorescent indicator fluo-4 was introduced in the basket cell interneuron through the patch-clamp pipette, at a concentration of 400 microM. Basket terminals were identified by co-localization of the two-photon basal fluorescence image of the presynaptic axon with the transmitted light image of the Purkinje cell soma. We compared the properties of the presynaptic action potential (AP) evoked calcium transients with those of the SCaTs obtained in the presence of ryanodine. Calcium transients were analyzed in terms of the percentage change in fluorescence with respect to the pre-stimulus period (% DF/Fo). In eight terminals, the calcium transients induced by a train of 4 APs (evoked by short intracellular depolarizations of the basket cell soma, at 50 Hz) had an average peak amplitude of 239 ± 153 % (mean ± s.d.). Their decay was approximated by a single exponential with average time constant of 3.6 ± 2.2 sec. In the same terminals, after block of APs by tetrodotoxin, ryanodine (5-10 microM) highly increased the frequency of SCaTs, suggesting that they are due to calcium release from intracellular stores, which is facilitated at this concentration of ryanodine (see Llano et al., Nature Neuroscience 2000). Single spontaneous events were characterized by a highly localized site of origin from which the signal diffused into the neighboring axonal regions. The relative change in fluorescence was measured for all pixels along the axonal tract and the source of the signal was identified as the site with the fastest rise. The amplitude and decay time were measured at the source. The average SCaT amplitude was 75 ± 16 %. The average decay (through an exponential fit) was 2.0 ± 0.8 sec. Larger events with a complex rise phase were often observed and could be attributed to high frequency bursts of summating single events. At each site of origin, SCaTs appeared to be clustered in time. We computed the cumulative probability for the inter-event-intervals (IEI) at each site, starting from the first event after ryanodine application. Most events (70%) had an IEI lower than 12 secs. Our data indicates that there can be spontaneous calcium transients at the very terminals of basket cell axons onto Purkinje cell somatas. These transients are due to intracellular store release of calcium, a mechanism different from the conventional calcium entry during APs invasion of the terminal; their amplitude can be higher than the latter and they could thus represent another form of vesicular release.