Electronic supplementary material (Hoppa et al.)

1 Skewed distribution of integrated P2X$_2$R currents Fig. 1c,d shows the histograms for the integrated currents (Q) of the events recorded at 0.2 and 2 μmol/l $[Ca^{2+}]_i$; non-Gaussian (skewed) distributions were obtained under both experimental conditions This is likely to be a consequence of the ATP content being proportionally related to the volume which in turn depends on the cube of the granule diameter. We have previously reported that the cubic root of the integrated current ($\sqrt[3]{Q}$) of the events recorded at low $[Ca^{2+}]_i$ exhibits a Gaussian distribution [1]. Simultaneous release of multiple granules will therefore *not* give rise to discrete peaks that are multiples of the unitary events regardless of whether the distribution of Q or $\sqrt[3]{Q}$ is displayed. In neurones, the amplitude distribution of the synaptic events likewise does not exhibit discrete peaks when the release probability is high [2].

2 Large events cannot be explained by non-resolved superimposition of many smaller events Our technique has sufficient temporal resolution to detect individual events that occurred within ~100 ms (Fig. 2c). The rate of exocytosis measured with the P2X$_2$R-based assay at 2 μmol/l $[Ca^{2+}]_i$ is 0.62 Hz [1]. If the frequency of the events is λ and the temporal resolution is t, then the probability P of the compound event that during time t at least two events occur is given by the expression

$$P = 1 - \exp(-\lambda t) - \lambda t \exp(-\lambda t).$$

We can thus estimate that the likelihood of *two* events occurring in such close succession that

they cannot be resolved is <0.002; for larger events (comprising ≥3 granules; Fig. 2), the likelihood is virtually zero.

3 Kinetics of SRB fluorescence increases The values of the rise times and half-widths observed in the SRB imaging experiments are much larger than those obtained in the $P2X_2R$-based assay (5-10 s rather than 5-10 ms; compare Fig 5e with Fig. 1f). It should be recognized, however, that whereas the $P2X_2R$ currents report the kinetics of ATP emptying of the granule halo surrounding the insulin core, the optical measurements reflect the entry of SRB into the granule lumen, much of which is occupied by a central insulin core (55% of the granule diameter; [3]). It is possible that the slow uptake of SRB might be related to the solvation of the central Zn^{2+}-insulin crystal. It is therefore interesting that fluorescence imaging indicates that the majority of Zn^{2+}-insulin crystal requires an average of 3.4 s to dissolve [4].

[1] Karanauskaite J, Hoppa MB, Braun M, Galvanovskis J, Rorsman P (2009) Quantal ATP release in rat beta-cells by exocytosis of insulin-containing LDCVs. Pflugers Arch 458: 389-401
[2] Edwards FA, Konnerth A, Sakmann B (1990) Quantal analysis of inhibitory synaptic transmission in the dentate gyrus of rat hippocampal slices: a patch-clamp study. J Physiol 430: 213-249
[3] Olofsson CS, Collins S, Bengtsson M, et al. (2007) Long-term exposure to glucose and lipids inhibits glucose-induced insulin secretion downstream of granule fusion with plasma membrane. Diabetes 56: 1888-1897
[4] Michael DJ, Ritzel RA, Haataja L, Chow RH (2006) Pancreatic beta-cells secrete insulin in fast- and slow-release forms. Diabetes 55: 600-607