Calcium-triggered fusion of synaptic vesicles and neurotransmitter release are fundamental signalling steps in the central nervous system. It is generally assumed that fast transmitter release is triggered by elevations in intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) to at least 100 \(\mu\)M near the sites of vesicle fusion\(^1\). For synapses in the central nervous system, however, there are no experimental estimates of this local [Ca\(^{2+}\)]\(_i\) signal. Here we show, by using calcium ion uncaging in the large synaptic terminals of the cockroach, that step-like elevations to only 10 \(\mu\)M [Ca\(^{2+}\)]\(_i\) induce fast transmitter release, which depletes around 80% of a pool of available vesicles in less than 3 ms. Kinetic analysis of transmitter release rates after [Ca\(^{2+}\)]\(_i\) steps revealed the rate constants for calcium binding and vesicle fusion. These show that transient (around 0.5 ms) local elevations of [Ca\(^{2+}\)]\(_i\) to peak values as low as 25 \(\mu\)M can account for transmitter release during single presynaptic action potentials. The calcium sensors for vesicle fusion are far from saturation at normal release probability. This non-saturation, and the high intracellular calcium cooperativity in triggering vesicle fusion, make fast synaptic transmission very sensitive to modulation by changes in local [Ca\(^{2+}\)]\(_i\).

The rapidly decaying, local [Ca\(^{2+}\)]\(_i\) signal that triggers vesicle fusion in presynaptic terminals cannot be readily be resolved with current imaging techniques. To circumvent this problem, we applied Ca\(^{2+}\) uncaging in large presynaptic terminals, the calyces of Held, to the brainstem medial nucleus of the trapezoid body. During Ca\(^{2+}\) uncaging, cytosolic [Ca\(^{2+}\)]\(_i\) is elevated in a spatially homogenous manner. Therefore, fluorescent Ca\(^{2+}\) indicators can be used to measure directly the biologically relevant [Ca\(^{2+}\)]\(_i\) acting at the sensor for vesicle fusion\(^9\).

Calyces were loaded through whole-cell patch pipettes with mixtures of the photolysable Ca\(^{2+}\) chelator DM-nitrophen (1 mM) and the low-affinity fluorescent Ca\(^{2+}\) indicator fura-2 FF (Fig. 1a). The transmitter release activity was monitored by recording excitatory postsynaptic currents (EPSCs) from principal neurons with a second patch pipette (Fig. 1a). Cyclothiazide (CTZ; 100 \(\mu\)M) was present during most recordings to suppress the desensitization\(^1\) of AMPA (\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-type glutamate receptors, which mediate fast EPSCs in these synapses.
We used flashes of varying intensities to study the magnitude of the postsynaptic response as a function of presynaptic \([\text{Ca}^{2+}]_i\). Dim flashes that elevated \([\text{Ca}^{2+}]_i\) to about 2 \(\mu\text{M}\) (Fig. 1b, blue trace) evoked small postsynaptic currents in which individual quantal events could be identified (Fig. 1c). Stronger flashes, which elevated the presynaptic \([\text{Ca}^{2+}]_i\), to roughly two- or threefold higher values (Fig. 1b, red and black traces), caused much larger EPSCs, in which individual quantal events were no longer detectable (Fig. 1d). Stable flash responses could be observed several times in a given cell pair. This is shown in Fig. 1d, in which the black and the grey trace, respectively, represent the response to the first and the seventh flash, which evoked similar presynaptic \([\text{Ca}^{2+}]_i\) changes and similar EPSCs. These large EPSCs reflect the release activity of several hundred active zones that operate in parallel at calyces of Held synapses.13,14

To quantify transmitter release rates, we deconvolved the multi-quantal EPSC responses with the quantal response waveform. This was necessary because peak EPSC amplitudes are not a valid measure of transmitter release, unless release is highly synchronized. We estimated the quantal response waveforms by recording spontaneous miniature EPSCs (mEPSCs) under the same conditions as flash-evoked EPSCs. Average mEPSC traces (Fig. 1f) had amplitudes of 33 ± 9.3 pA, and their decay was biphasic, with fast and slow time constants of 1.9 ± 0.4 and 13.4 ± 2.6 ms, respectively (\(n = 5\) cells). The deconvolution of flash-evoked, compound EPSCs therefore assumed such quantal response waveforms with double-exponential decays (see Methods). Deconvolution revealed peak release rates of about 10 and 60 quanta per ms (see Fig. 1e) for the flash-evoked, multi-quantal EPSCs shown in Fig. 1d.

In experiments with a higher DM-nitrophen concentration (2 mM), step-like elevations of up to 25 \(\mu\text{M} \,[\text{Ca}^{2+}]_i\) were produced (see Fig. 1g), which evoked larger EPSCs with faster rise times. EPSCs reached maximal amplitudes at \([\text{Ca}^{2+}]_i\) \(\approx 12 \, \mu\text{M}\). Increasing \([\text{Ca}^{2+}]_i\), further caused a speeding of the rising phase, but did not alter the amplitude of EPSCs (compare black and red traces in Fig. 1h). The corresponding integrated release rate traces (Fig. 1i, dashed lines) show that at \([\text{Ca}^{2+}]_i > 12 \, \mu\text{M}\), cumulative release was similar within around 3 ms after the onset of release, indicating that a pool of readily releasable vesicles may have been depleted by such \([\text{Ca}^{2+}]_i\) steps. The late (>3 ms) slow rise in the integrated release rate traces may be due to inaccuracies in the release rate estimates during the decaying phase of EPSCs (Fig. 1h, i) or to a genuine slow component of transmitter release. A small effect of postsynaptic receptor saturation on the peak EPSC amplitudes of the largest responses cannot be excluded. This, however, should not affect our estimate of peak release rates, as these occur at the point of the steepest rise of EPSCs, before the EPSCs reach their peak amplitudes (Fig. 1h, i).

We next tested whether \(\text{Ca}^{2+}\) uncaging and the \([\text{Ca}^{2+}]_i\) signal created by the opening of voltage-gated \(\text{Ca}^{2+}\) channels induce vesicle fusion from a common pool of readily releasable vesicles. We studied the inhibition of depolarization-induced EPSCs by preceding flash-evoked transmitter release (Fig. 2). In the example shown in Fig. 2, a depolarization-evoked \(\text{Ca}^{2+}\) current induced a control EPSC of 25.8 nA (Fig. 2b, black trace), which was reduced to 18.1 nA (Fig. 2b, blue trace) and 4.4 nA (Fig. 2b, red trace) when the depolarizing pulses were preceded by flashes of varying intensities. This inhibition was not accompanied by a reduction in presynaptic \(\text{Ca}^{2+}\) currents (\(i_{\text{Ca}}\) was \(97 \pm 5\%\) of control after flashes; \(n = 9\)). Half-maximal and 80% inhibition occurred with \([\text{Ca}^{2+}]_i\) steps of around 10

![Figure 1](https://example.com/image1.png)

**Figure 1** Presynaptic \([\text{Ca}^{2+}]_i\) steps and postsynaptic detection of transmitter release. a, Fluorescent images at 380 nm excitation of a calyx of Held nerve terminal filled through a whole-cell patch pipette with \(\text{Ca}^{2+}\)-indicator, fura-2 FF and photoconvertible \(\text{Ca}^{2+}\)-chelator, DM-nitrophen (1 mM). Red dots indicate binned pixels used for the analysis of cellular fluorescence changes. b, Elevation of presynaptic \([\text{Ca}^{2+}]_i\), obtained by light flashes with different transmission intensities. c, EPSCs in response to the presynaptic \([\text{Ca}^{2+}]_i\) elevations shown in b. Colours identify corresponding \([\text{Ca}^{2+}]_i\) traces in b-e. e, Transmitter release rates obtained from deconvolution of two EPSCs shown in d, f. An averaged mEPSC trace from 436 individual traces. g-i, Presynaptic \([\text{Ca}^{2+}]_i\) elevations (g), EPSCs (h) and transmitter release rates (i) for a different cell pair. In i, cumulative release rates (dashed lines) were obtained by integrating the corresponding release rate traces.

![Figure 2](https://example.com/image2.png)

**Figure 2** \([\text{Ca}^{2+}]_i\) elevations induced by flashes and presynaptic depolarizations release vesicles from a common pool. a, Presynaptic \(\text{Ca}^{2+}\) currents \((i_{\text{Ca}})\) under control conditions (black trace) and after a flash (red trace). \(\text{Ca}^{2+}\) currents were evoked by a 4-µs depolarization to +80 mV, followed by a 16-ms period at 0 mV. b, EPSCs corresponding to the traces shown in a. The blue and red traces were obtained after flashes that elevated the presynaptic \([\text{Ca}^{2+}]_i\) to the indicated levels. c, Inhibition of depolarization-evoked EPSCs as a function of flash-induced presynaptic \([\text{Ca}^{2+}]_i\). Half-maximal inhibition occurred at \(\sim 7 \, \mu\text{M} \,[\text{Ca}^{2+}]_i\), as indicated by a Hill-function fit of the data (solid line). d, Cumulative transmitter release, analysed for \(n = 10\) cells by integrating the release rates in a 10-ms interval (Fig. 1). The predictions of the model shown in Fig. 3e are superimposed (solid line).
7 and 10 μM, respectively (Fig. 2c). Figure 2d shows the \([Ca^{2+}]_i\) dependence of cumulative transmitter release, obtained by integrating the release rates over the first 10 ms after flashes (see Fig. 1i). The inhibition of depolarization-induced EPSCs (Fig. 2c) and the cumulative transmitter release (Fig. 2d) showed almost identical \([Ca^{2+}]_i\) dependencies. This indicates that both types of \(Ca^{2+}\) stimulus draw upon the same pool of rapidly releasable (<10 ms) vesicles. Cumulative release reached a plateau of 1,785 ± 870 released quanta with \([Ca^{2+}]_i\) steps above 12 μM (n = 8 flashes; see Fig. 2d). This number is larger than a previous pool size estimate based on analysing EPSC charges in the absence of CTZ, perhaps owing to a contribution of AMPA-receptor desensitization in the previous study. Nevertheless, the pool size estimates showed considerable scatter between cells (range 800–3,500; see Fig. 2d).

Figure 3a shows the \([Ca^{2+}]_i\) dependence of transmitter release rates, measured from 47 flash responses in 13 cell pairs. In the range 2–8 μM \([Ca^{2+}]_i\), peak release rates increased as a power function of \([Ca^{2+}]_i\), with an exponent of 4.2, as indicated by linear regression analysis in the double-logarithmic plot of Fig. 3a. This exponent is similar to previous values obtained by varying the extracellular \(Ca^{2+}\) concentration at neuromuscular junction synapses or at central synapses, including the calyx of Held. At values above 10 μM, release rates increased with a more shallow dependency on \([Ca^{2+}]_i\). In the range 8–13 μM \([Ca^{2+}]_i\), the average release rate was 349 ± 209 ms⁻¹ (n = 9 flashes), a value similar to the peak release rates measured during action-potential-evoked EPSCs (see below; Fig. 4). Nevertheless, the corresponding average \([Ca^{2+}]_i\), (10.7 ± 1.5 μM) is not necessarily equal to the peak \([Ca^{2+}]_i\), reached at the \(Ca^{2+}\) sensors for vesicle fusion during a single presynaptic action potential. This is because the local \([Ca^{2+}]_i\) signal during an action potential probably decays very rapidly, which might curtail transmitter release before \(Ca^{2+}\) binding to the sensor for vesicle fusion reaches equilibrium.

To learn about the rate-limiting steps involved in \(Ca^{2+}\)-dependent vesicle fusion at the calyx of Held synapse, we fitted the flash-photolysis data with a minimal kinetic model, incorporating several \(Ca^{2+}\)-binding steps, followed by vesicle fusion from the fully \(Ca^{2+}\)-bound state (Fig. 3e). As it has been reported that uncaging of \(Ca^{2+}\) by flash photolysis can, under certain conditions, lead to brief (~1–2 ms) spike-like overshoots of \([Ca^{2+}]_i\), that would have been undetected by \(Ca^{2+}\) imaging, we first modelled the expected \([Ca^{2+}]_i\) time course after flash photolysis (see Methods). The resulting \([Ca^{2+}]_i\), time course (Fig. 3d) did not show a significant spike. This is attributable to the almost full loading of DM-nitrophen with \(Ca^{2+}\), and to rapid \(Ca^{2+}\) buffering by ATP and by endogenous Ca buffers. The calculated \([Ca^{2+}]_i\), time course was then used to drive the \(Ca^{2+}\)-binding scheme shown in Fig. 3e. To predict the high initial slope of 4.2 in the double-logarithmic plot of release rates versus \([Ca^{2+}]_i\), (Fig. 3a), it was necessary to include five \(Ca^{2+}\)-binding steps, as well as a cooperativity factor \(b\) in the scheme of Fig. 3e.

The analysis of synaptic delays (Fig. 3b) and times to peak release (Fig. 3c) gave important constraints for setting the \(Ca^{2+}\)-binding and -unbinding rate constants. When these rate constants were increased, a good fit of the peak release rates (Fig. 3a) was still obtained, but the delays and times to peak release (Fig. 3b, c) were then significantly underestimated at low \([Ca^{2+}]_i\) (data not shown). The set of parameters found by fitting the data in Fig. 3 also accurately predicted the decrease in cumulative transmitter release with submaximal \([Ca^{2+}]_i\), elevations (Fig. 2d). Note, however, that the value of the vesicle fusion rate \(\gamma\) is only a lower limit estimate because the finite rising speed of mEPSCs, as well as possible postsynaptic receptor saturation, might contribute to the apparent levelling-off of peak release rates at \([Ca^{2+}]_i\) > 10 μM.

Knowledge of the kinetic parameters of \(Ca^{2+}\) binding and vesicle fusion (Fig. 3) should allow us to infer the time course and amplitude of the local \([Ca^{2+}]_i\), signal during transmitter release

![Figure 3 Intracellular Ca²⁺ concentration dependence of transmitter release rates at the calyx of Held synapse.](image-url)

![Figure 4 The predicted local [Ca²⁺] signal at an average Ca²⁺ sensor for vesicle fusion during single presynaptic action potentials.](image-url)
triggered by presynaptic action potentials. To do so, we first measured transmitter release rates during EPSCs evoked by afferent fibre stimulation. Six consecutive EPSCs, obtained in the presence of 100 μM CTZ, are shown in Fig. 4a on an expanded time scale. The addition of CTZ to the bath solution increased peak EPSC amplitudes by 17.7 ± 11.1% (n = 4), an effect that is probably caused by the more effective peak summation of mEPSCs with slowed decay times in the presence of CTZ (but see refs 16 and 20). The EPSCs were deconvolved with qantum response waveforms, which were obtained by sampling spontaneous mEPSCs between single fibre stimulations for each cell. The average peak release rate was 335 ± 11 ms⁻¹ (n = 4 cells). The duration of transmitter release at half-maximal amplitude was 0.44 ± 0.07 ms (Fig. 4b), in good agreement with a previous estimate obtained from delay histograms 11.

We next set out to calculate the local [Ca²⁺], signal that an average vesicle might experience during a presynaptic action potential. As a starting point we assumed this signal to be proportional to the Ca²⁺ current measured during action potentials at the calyx of Held13. This implies that the local [Ca²⁺], transient is produced by overlap of [Ca²⁺], domains created by several neighbouring Ca²⁺ channels within the release zone. We then performed model simulations with the kinetic parameters obtained from the flash data (Fig. 3), and varied the decay and the amplitude of the local [Ca²⁺], waveform to obtain adequate predictions of transmitter release during action potentials. In four cells with average EPSC amplitudes of 5.88 ± 4 nA, we found [Ca²⁺], transients with peak amplitudes of 27.8 ± 1.4 μM and half-widths of 0.46 ± 0.05 ms. The width at half-amplitude of the inferred local [Ca²⁺], signal exceeded that of presynaptic [Ca²⁺] currents during action potentials13 by only ~100 ms (Fig. 4b, compare solid and dashed red lines), indicating that fast processes such as diffusion or binding to endogenous Ca²⁺ buffers11 must be extremely effective in terminating the local [Ca²⁺], signal after Ca²⁺ channels close.

We have shown that the fast phase of transmitter release at a central nervous system (CNS) synaptic terminal occurs at [Ca²⁺], values that are significantly lower than the range of 75–300 μM predicted by theoretical studies11,5, by experiments on invertebrate synapses12,19 or by flash photolysis in retinal bipolar cell terminals13. In ref. 4, secretion measured by membrane capacitance was absent below 10–20 μM [Ca²⁺], but see ref. 21, a Ca²⁺ concentration at which we observe strong transmitter release from calyces, at rates equalling those obtained with single action potentials (Fig. 3a). It is possible that ribbon-type synapses found in sensory systems have vesicle fusion mechanisms with a lower Ca²⁺ sensitivity than those at the calyx of Held synapse with its conventional active zones. It is usually assumed that fast synaptic transmission is driven by rapid local [Ca²⁺], transients to values above 100 μM, which might be found in the immediate vicinity (<10 nm) of an open Ca²⁺ channel11,13. Our data show that fast vesicle fusion can occur at much lower peak [Ca²⁺], during single action potentials (~25 μM; Fig. 4b), indicating that tight (<10 nm) molecular coupling of vesicles and Ca²⁺ channels at this synapse is not necessary for fast transmitter release. This conclusion is supported by the finding that presynaptic injection of EGTA, a Ca²⁺ buffer with a slow binding rate, reduces EPSC amplitudes at this13 and at neocortical synapses22, again implying that a significant contribution to the local [Ca²⁺], signal originates from remote channels. The possibility that the colocalization of vesicles and Ca²⁺ channels becomes more tight with maturation of synapses can, however, not be excluded at present. In any case, the local [Ca²⁺], at the sensors for vesicle fusion is about fifty times higher than the spatially averaged [Ca²⁺], attained with single action potentials (~0.5 μM)13, confirming the significance of spatially localized [Ca²⁺], signals for synaptic transmission11,5.

Action potentials released 178 ± 86 quanta (n = 4 cells) within 2 ms (Fig. 4), corresponding to roughly 10% of the pool of readily releasable vesicles as determined in Fig. 2. This rather small released fraction, which is at the lower limit of previous estimates at this synapse14,17, is a typical feature of CNS synapses13. Sustained [Ca²⁺], elevations to 25 μM (Fig. 1g, h, i; black traces) lead to transmitter release rates several times larger than those observed with single presynaptic action potentials (Fig. 4b), indicating that most Ca²⁺ sensors do not reach the fully occupied state during single presynaptic action potentials. This conclusion agrees with the finding at this synapse11 that increasing the extracellular Ca²⁺ concentration leads to marked potentiation of EPSC amplitudes. The non-saturation of the Ca²⁺ sensors during an action potential and the high intracellular Ca²⁺ cooperativity of vesicle fusion (Fig. 3a) render small changes in amplitude or time course of the local [Ca²⁺], signal very effective in modulating the amount of transmitter output at a synapse. Such changes in the local [Ca²⁺], signal are probably involved in presynaptic inhibition of transmitter release12 as well as in Ca²⁺- and activity-dependent forms of synaptic plasticity18.

**Note added in proof:** A similar relationship between transmitter release and [Ca²⁺], has recently been reported using Ca²⁺ uncaging by laser photolysis19.

**Methods**

**Tissue preparation**

We prepared transverse brainstem slices 200 μm thick from 8–10-day-old Wistar rats13,14. Whole-cell voltage-clamp recordings were done with a double patch-clamp amplifier (EPC-9/2, HEKA elektronik) from presynaptic calyces and postsynaptic principal neurons. The holding potential was −80 mV, and pre- and post-synaptic series resistances were compensated up to 80%. For action potential stimulation of calyces, only the postsynaptic cell was recorded (Fig. 4), and afferent fibres were stimulated with a bipolar electrode. The extracellular solution was a standard Ringer solution with 2 mM CaCl₂ and 1 mM MgCl₂ to which 10 mM tetraethylammonium (TEA), 50 mM 2-amino-5-phosphonovaleric acid and 100 μM CTZ were added during paired recordings to suppress undesired current components and desensitization of AMPA receptors13,15. Postsynaptic pipette (intracellular) solutions were as described20. Presynaptic pipette solutions were made by stocks containing (final concentrations in mM) 130 Glucose, 20 TEA-Cl, 20 HEPES, 5 Na₂ATP and 0.1 Na₂GTP, to which 2 mM (Tfela; 0.1 mM) DM-nitrophen (Calbiochem; 1 or 2 mM), CaCl₂ (0.85 or 1.7 mM) and MgCl₂ (0.5 or 0.8 mM) were added. For control experiments at 1 mM free intracellular Mg²⁺ (Fig. 3a), 0.1 mM MgCl₂ was added. 2 mM NP-EGTA (Molecular Probes), 4 mM CaCl₂, 3.3 mM MgCl₂, 2 mM Na₂ATP and 0.3 mM Na₂GTP were added. Experiments were done at room temperature (21–24 °C). Results are reported as mean ± s.d.

**Ca²⁺ uncaging and imaging**

We used single light pulses (~1.5 ms) from a flash lamp (Rapp Optoelektronik) for photolysis. (Ca²⁺)⁺ imaging was rescued 4 ms after the flash trigger (Fig. 1b), by exciting fura-2 FF at 350 nm and 380 nm with a monochromator (TILL-photons). Pixel binning (8 x 8; see Fig. 2c) was used to reduce the exposure times (5 ms), and free [Ca²⁺], was calculated from background-corrected fluorescence ratios of a series of pixels in the calyceal layer. The dissociation constant (10 ± 2 μM; n = 5) of the fura-2FF-Ca²⁺ complex was first determined in vitro, by using mixtures of CaCl₂ and Ca²⁺ buffers (DM-nitrophen plus DPTA or HEGTA). We obtained the calibration constants at nominally 0 Ca²⁺ by loading calyces with 150 mM EGTA, 0 CaCl₂ and 0.2 mM MgCl₂ (R₀EGTA) by loading calyces with calibration solutions (n = 4 each). For R₀EGTA, the pipette solution contained 10 μM Ca²⁺ (as CaCl₂), buffered by isosomolar DPTA (80 mM), and the extracellular solution was a Na²⁺-free Ringer with 10 μM CaCl₂. R₀EGTA was taken at the end of a 200-s recording period at a holding potential of 0 mV. The ratio at a saturating [Ca²⁺] signal (Rₛaturating; 10 μM CaCl₂) was taken from in vitro measurements. The calibration constants were corrected11 for changes in DM-nitrophen fluorescence27 after flashes.

**Decovolution analysis**

EPSC current traces were low-pass filtered at 6 kHz and digitized at 20 kHz. Residual series resistance (1.5–3 MD) after partial electronic compensation was compensated off-line8 before display and analysis. This led to increased in current amplitudes of up to 50%, and to small decreases in the rise times of fast EPSCs (Fig. 4). We calculated transmitter release rates from the EPSC and its time derivative according to the equation given in ref. 15, extended by a term for the slowly decaying mEPSC component (E.N. & T. Sakaba, manuscript in preparation). The mEPSC amplitude, the fast and slow decay time constants and the relative contribution of the slow component were set to the average values found from fits of mEPSCs (see text). This deconvolution analysis provides release rates averaged over time windows equal to the time-to-peak of mEPSCs. It assumes that mEPSCs add up linearly; this assumption might be violated in synapses with strong effects of glutamate spillover10. However, a detailed analysis (E.N. & T. Sakaba, manuscript in preparation) shows that the effects of build-up of residual glutamate should be small decreases in the rise times of fast EPSCs (Fig. 4b). Before display and analysis. This led to increased in current amplitudes of up to 50%, and to small decreases in the rise times of fast EPSCs (Fig. 4). We calculated transmitter release rates from the EPSC and its time derivative according to the equation given in ref. 15, extended by a term for the slowly decaying mEPSC component (E.N. & T. Sakaba, manuscript in preparation). The mEPSC amplitude, the fast and slow decay time constants and the relative contribution of the slow component were set to the average values found from fits of mEPSCs (see text). This deconvolution analysis provides release rates averaged over time windows equal to the time-to-peak of mEPSCs. It assumes that mEPSCs add up linearly; this assumption might be violated in synapses with strong effects of glutamate spillover10. However, a detailed analysis (E.N. & T. Sakaba, manuscript in preparation) shows that the effects of build-up of residual glutamate should be small decreases in the rise times of fast EPSCs (Fig. 4b).
Kinetic simulations

The \([Ca^{2+}]\), time course with flashes (Fig. 3d) was simulated from the measured time course of the flash by numerical integration of a system of differential equations for the implicated Ca\(^{2+}\) and Mg\(^{2+}\) buffers. We assumed the presence of an endogenous Ca\(^{2+}\) buffer with binding capacity \(a\) of 40, as determined experimentally at calyces of Held\(^{12}\). The binding rate constants of DM-nitrophen, ATP and endogenous Ca\(^{2+}\) buffer were as in ref. 29. A different set of binding rate constants for the endogenous Ca\(^{2+}\) buffer at constant \(a\) of 40 \(\mu M\) exhibited similar results (data not shown). The \([Ca^{2+}]\) time course of Fig. 3d was used to drive the kinetic scheme for Ca\(^{2+}\) binding and vesicle fusion (Fig. 3e), which was solved numerically. Release rates were obtained by differentiating the accumulation of vesicles in the fused state, assuming a pool of 1,800 vesicles (Fig. 2d).

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Figure 1 Measles virus binding to CHO cells expressing SLAM or CD46. CHO cells were transiently transfected with pCAGGS, pCAGSLAM or pCAGCD46. The efficiency of transfection was usually more than 50% as determined by flow cytometry analysis. The transfected cells were incubated with the PKK (a) or Edmonston (b) strain of MV for 30 min. After washing, the cells were incubated with a monoclonal antibody specific for MV H protein and stained with FITC-labelled anti-mouse IgG.

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Measles virus continues to be a major killer of children, claiming roughly one million lives a year. Measles virus infection causes profound immunosuppression, which makes measles patients susceptible to secondary infections accounting for high morbidity and mortality. The Edmonston strain of measles virus, and vaccine strains derived from it, use as a cellular receptor human CD46 (refs 3, 4), which is expressed on all nucleated cells; however, most clinical isolates of measles virus cannot use CD46 as a receptor. Here we show that human SLAM (signalling lymphocyte-activation molecule; also known as CD150), a recently discovered membrane glycoprotein expressed on some T and B cells, is a cellular receptor for measles virus, including the Edmonston strain. Transfection with a human SLAM complementary DNA enables non-susceptible cell lines to bind measles virus, support measles virus replication and develop cytopathic effects. The distribution of SLAM on various cell lines is consistent with their susceptibility to clinical isolates of measles virus. The identification of SLAM as a receptor for measles virus opens the way to a better understanding of the pathogenesis of measles virus infection, especially the immunosuppression induced by measles virus.

A marasmotic B-cell line transformed with Epstein–Barr virus (EBV), B95–8, and its adherent subline B95a are highly sensitive to measles virus (MV) present in clinical specimens; and strains isolated in B95a cells, but not in Vero (monkey fibroblast) cells, retain pathogenicity for monkeys. Thus, B95a is now commonly used to isolate MV from clinical specimens. Unlike the Edmonston strain, which is capable of infecting all CD46+ primate cell lines, MV strains isolated in B95a or other human B-cell lines infect only some primate B-cell and T-cell lines. By using vesicular stomatitis virus (VSV) pseudotypes bearing MV envelope proteins, we showed that virus entry is a principal determinant of cell tropism of MV strains. Thus, the receptor molecule that enables B95a-isolated MV strains to enter cells appears to be present on a restricted number of lymphoid cell lines. To identify this receptor, we carried out functional expression cloning using the pseudotype system in which the recombinant VSV containing the green fluorescent protein (GFP) was used to drive the kinetic scheme for Ca\(^{2+}\) binding and vesicle fusion (Fig. 1).

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