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Amperometric measurements at cells support a role for dynamin in the dilation of the fusion pore during exocytosis

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Abstract

Dynamin is a GTPase mechanochemical enzyme involved in the late steps of endocytosis, where it separates the endocytotic vesicle from the cell membrane. However, recent reports have emphasized its role in exocytosis. In this case, dynamin may contribute to the control of the exocytotic pore, thus suggesting a direct control on the efflux of neurotransmitters.

Dynasore, a selective inhibitor of the GTPase activity of dynamin, was used to investigate the role of dynamin in exocytosis. Exocytosis was analyzed by amperometry, thus revealing that dynasore inhibits exocytosis in a dose-dependent manner. Analysis of the exocytotic peaks showed that the inhibition of the GTPase activity of dynamin led to shorter, smaller events. This observation, together with the rapid effect of dynasore, suggests that the blocking of the GTPase induces the formation of a more narrow and short-lived fusion pore.

These results suggest that the GTPase properties of dynamin are involved in the duration and kinetics of exocytotic release. Interestingly, and in strong contrast with its role in endocytosis, the mechanochemical properties of dynamin appear to contribute to the dilation and stability of the pore during exocytosis.

Keywords

single cell amperometry; dynamin; exocytosis; dopamine; fusion pore

1. Introduction

Exocytosis is the fundamental phenomenon enabling neuronal communication. During this event, typically thought to occur at the synaptic end of the axon, a vesicle filled with neurotransmitters, such as dopamine, fuses with the cell membrane.^[1] This fusion results in the formation of a pore, and the release of the vesicular content into the synapse. These molecules then affect post-synaptic receptors, eventually altering the transmission of the neuronal signal.

The formation of the fusion pore, and its dynamics, are important in the regulation of exocytosis. These parameters can control and regulate the signal by tuning the vesicular efflux or the amount of molecules released. The initiation of the fusion pore is triggered by the SNARE proteins. These proteins, present on the vesicles and the intracellular side of the

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cell membrane, form a complex upon stimulation and trigger the fusion of the lipid membranes of the cell and the vesicle, thus forming the pore (see Figure 1). The geometry of this pore controls the flux of neurotransmitters, following Fick's laws of diffusion.^[2] The fate of this fusion pore is still being debated in the literature. The 'full distension' paradigm assumes that the vesicle releases all its content and is then integrated into the cell membrane, as shown on Figure 1. Recent findings, showing that PC12 cell vesicles release only 40 % of their content,^[3] actually indicate that the pore closes again before all the vesicular content has been released, and that the vesicle is probably then most often used again. This phenomenon is known as extended 'kiss and run'.^{[4],[5]}

Dynamin is a GTPase mechanochemical enzyme which can self-assemble and form a helicoidal complex.^[6] Dynamin spontaneously forms rings and helical spirals onto negatively charged lipid nanotubes and liposomes. Dynamin can also bind to phosphatidylinositol-4,5-bisphosphate (PIP₂) rich lipid membranes.^[7] These structures constrict and vesiculate upon addition of GTP.^[8] Dynamin is involved in endocytosis, where it contributes to the closing of the neck of the newly formed vesicle and to separate it from the cell membrane. Recent reports suggest a possible role for dynamin in exocytosis.^{[9],[10]} Using near-field fluorescence microscopy, it was found that dynamin might control the dilation of the pore, and contribute to the discrimination between full distension and kiss-and-run.^[10] Similarly, the inhibition or depletion of dynamin 2 was found to reduce the cytotoxic activity of NK cells^[11] indicating that dynamin 2 regulates granule secretion in these cells.

Single cell amperometry has been used since the early 90s to study exocytosis.^{[12]–[14]} In this method, a carbon fiber microelectrode is positioned against the cell, and held at a potential sufficient to oxidize neurotransmitters.^{[15],[16]} When exocytosis is triggered, the released transmitters are detected at the electrode in a diffusion limited manner. Single exocytotic events can be detected, leading to the recording of a peak, whose characteristics are controlled by the geometry of the pore,^{[2],[4],[17]} the microenvironment,^[42] and the physical or pharmacological conditions the cell has been exposed to.^{[18]–[21]} This technique allows the real-time, quantitative study of exocytosis. Precise analysis of pre and post spike exocytotic features can be carried out owing to the high temporal resolution, and it has been shown that exogenous lipids can alter release kinetics.^[4] The effect of lipids suggests that the biophysics of the lipid membrane is involved in the dynamics of the fusion pore.^{[4],[22],[23]}

In this report, we use single cell amperometry to investigate the effect of blocking dynamin action on exocytosis. Dynasore, a selective, non-competitive blocker of the GTPase activity of dynamin, has been used.^{[24],[27]} Interestingly, it is reported that dynasore does not impair the lipid binding capability of dynamin or its self-assembly. The inhibition of dynamin decreases, and ultimately completely abolishes, the exocytotic activity of PC12 cells. Peak analysis^{[25],[26],[28]} reveals that the presence of dynasore leads to shorter, smaller peaks, which is an indication of a narrow and short-lived fusion pore. Examining the decaying slope of the peak indicates that the pore might be more likely to close during the course of the exocytotic release when dynamin is inhibited. From these results, and in agreement with the previously reported results, it is suggested that dynamin forms a structure that supports and frames the pore, possibly contributing to the observation of larger exocytotic events.

2. Results and Discussion

2.1. Dynasore reduces cell exocytotic ability in a dose dependent manner

PC12 cells were incubated for 5-10 min in HEPES buffer containing different concentrations of dynasore. Increasing concentrations of dynasore were found to inhibit the

probability that a cell shows some exocytotic capability (*i.e.* that at least one peak is recorded). Figure 2 presents the fraction of cells generating at least one exocytotic peak over the number of cells tested as a function of the dynasore concentration (on a logarithmic scale). The graph shows a clear inhibitory effect, as less cells respond to stimulation in presence of high concentrations of dynasore. The dose-response curve has been fit with a sigmoid and c_{50} , the concentration dynasore leading to a 50% decrease in activity compared to control, was 1.6 μM . This result indicates that dynamin activity is involved in exocytosis, in agreement with previous reports,^{[9],[10]} and is involved in promoting exocytosis.

2.2. Increasing concentrations of dynasore lead to shorter and faster peaks

The events obtained from the control treatments and from the cells incubated with 1 μM and 100 nM dynasore were selected for peak analysis. Several parameters were considered as outlined in Figure 3 (see the Experimental Section for a description of these parameters). Figure 4 shows two average peaks obtained from typical traces, for the control and 1- μM dynasore treatments. The exposure to dynasore, and, hence, inhibition of the GTPase activity of dynamin, leads to narrower peaks. Additionally, the magnitude of each peak is lower, and the amount of molecules released is decreased in comparison to control. Considering that the cells were pre-incubated with dynasore for only 5-10 min, a reduction of vesicular content is very unlikely. For instance, cells treated with reserpine, a potent inhibitor of the vesicular monoamine transporter, do not show a decreased N until after at least an hour of incubation.^[3] The short incubation time therefore indicates that the lipid pore dynamics, rather than the vesicle properties, are altered. In particular, the low, sharp peaks observed after exposure to dynamin, appear to indicate a narrow, short-lived fusion pore.

The numerical values obtained from the control treatments, and from the cells incubated with 1 μM and 100 nM dynasore are summarized in Table 1. The distribution of the exocytotic parameters is asymmetric and strongly deviates from normality, hence, motivating the use of the median in place of the mean.^{[14],[17],[28],[29]} These data reveal that inhibition of dynamin GTPase activity leads to significant decreases in the characteristic peak times, $t_{1/2}$ and t_{fall} , in the peak current i_p and in the released number of molecules, N . Interestingly, this effect is more important when the concentration of dynasore increases. The lower peak current suggests that the radius of the lipid pore is reduced by dynasore. This is a strong indication that dynamin GTPase activity is involved in opening this structure. Additionally, the shorter characteristic times, leading to a decreased amount of released neurotransmitters, hints that the pore stays open for a shorter time than for the control case. This finding is in good agreement with the idea that the vesicle partially releases (about 40%) its content during exocytosis^[3], and that the pore closes again before the complete depletion of the vesicular content.^[4]

The effect of dynasore has been tested against several other GTPases, and has been found to be highly selective towards dynamin.^[24] This selectivity emphasizes the role of dynamin in exocytotic release. However, other proteins, such as the SNARE (soluble NSF-attachment protein receptors) proteins could be involved. The SNARE proteins initiate vesicular fusion, and thus partially control the dynamics of the pore during exocytosis.^[30] Cleavage of these proteins with botulinum neurotoxins^{[31],[32]} or silencing with siRNAs^[33] leads to a smaller occurrence of exocytotic events, without any alteration of the peak shape. Furthermore, deletion of the 9 C-terminal residues of the SNAP-25, inducing a looser zipping of the SNARE complex, has been shown to lead to broader amperometric spikes, without any significant change in the amount of molecules released.^[34] The differences between these previous reports and our data suggest that dynasore does not hinder the formation of the SNARE complex, and that its activity can be mostly attributed to the inhibition of the GTPase activity of dynamin.

Furthermore, blocking of dynamin with anti-dynamin immunoglobulins was found, up to 10 min after the treatment, to induce fewer peaks, and to reduce the peak magnitude.^[35] The short incubation time guarantees that the observed effect is not due to the inhibition of endocytosis, leading reduced catecholamine recycling and to vesicle depletion. However, after longer incubations (22 min), the observed spikes are larger than the ones observed with the control treatments.^{[35],[36]} The exocytotic ability of the cell then fades off, indicating that the vesicular pool is completely depleted because of the inhibition of endocytosis. The differences between the results obtained with dynasore and anti-dynamin are expected to be due to the different activities of these inhibitors. Dynasore is expected to block the GTPase activity of dynamin, without altering its self-assembly or its lipid binding activity.^[24] The anti-dynamin antibodies might inhibit the self-assembly of dynamin, or its binding to the lipid membrane, thus leading to a different response from that observed here.

Our results suggest inhibition of dynamin GTPase activity by dynasore results in a more constrained vesicular efflux. Following exposure to dynasore the pore is narrower and less stable (*i.e.* stays opened for a shorter duration).

2.3. Inhibition of dynamin GTPase activity increases the prevalence of double exponential decays

The peak parameters shown in Table 1 show that exposure to increasing concentrations of dynasore induces a decrease in t_{fall} . Assuming that the initial vesicular content is not affected by the short (5-10 min) dynasore pre-incubation, this observation strongly suggests that this decay arises from the closing of the pore, which is faster in presence of dynasore. This observation agrees well with the recent description of post-spike feet, probably generated by the closing of the lipid pore formed by the vesicle and the membrane.^[4]

Several reports have highlighted that the current decay following the peak of the exocytotic event can be fit either with a single or a double exponential.^{[28],[37]} This fact suggests different sub-populations of pores, which may be induced by variations in $[Ca^{2+}]$ levels, number of SNARE complexes, or synaptotagmin interactions.^[34] Recent numerical models of exocytosis reveal that, in the case of an expanding pore, the current decay is better fitted with a single exponential, regardless of the rate of opening of the pore.^[38] In this case, the asymptotic decay of the exocytotic current is mostly controlled by diffusion of the neurotransmitters through the fusion pore. As a consequence, a perfect single exponential asymptotic behavior strongly hints that the pore does not close again during the course of the exocytotic release, or at least that it does not close at a rate faster than the diffusional depletion of the vesicle. Based on these considerations, the decay of all the peaks observed in our experiments was fit with a double decaying exponential,

$$f(t) = A_1 e^{-\frac{t-t_0}{T_1}} + A_2 e^{-\frac{t-t_0}{T_2}} \quad (1)$$

where t_0 is the starting time of the decay, and T_1 and T_2 are the characteristic decay times (with $T_1 < T_2$). If a single exponential provides a better fit, a criterion would be that $T_1 \approx T_2$. Figure 5A shows a scatter plot of T_1 vs. T_2 obtained from the control peaks. A clear sub-population, centered over the identity function $f(x) = x$, can be observed, indicating that a significant fraction of the peak decays satisfies the ' $T_1 \approx T_2$ ' criterion and are better fit with a single exponential.

To evaluate if the decay is better fit with a single or double exponential the normalized distance d between each point (T_1, T_2) and the line describing the identity function was examined (Figure 5B). If d is small, then the ' $T_1 \approx T_2$ ' condition is satisfied and this peak is better fit with a single exponential. The normalized distance d was calculated as follows,

$$d(T_1, T_2) = \frac{T_2 - T_1}{\sqrt{2} \| (T_1, T_2) \|} = \sin(\theta) \quad (2)$$

where $\| (T_1, T_2) \|$ is the norm of the vector (T_1, T_2) , and θ is the angle formed by the vectors (T_1, T_2) and $(1, 1)$. This parameter was calculated for every peak. The results obtained for the control and 1 μM dynasore treatments are presented on Figure 5C. In this cumulative histogram, the y-axis shows the calculated d as a function of the fraction of the peak population with value below this d . For instance, about 45% of the peaks show a d below 0.1 for the control treatment. Thus these peaks are better fit with a single exponential using Equation 1 and shown in Figure 5C.

Interestingly, a good fit to these curves is a sigmoid. The plateau at the top of each curve represents points best fit with a double exponential decay as modeled from Equation 1. The shape of these curves shows a higher prevalence of double exponential decays for the dynasore-treated cells, as indicated by the leftward shift of the inflection point of the sigmoid (control: 58%; 100 nM dynasore: 52%; 1 μM dynasore: 44%). This fact suggests that the inhibition of dynamin GTPase activity increases the probability of observing a peak generated by a fusion pore closing (constricting) at a rate faster than the diffusional clearance of amine from the vesicle. Thus, in good agreement with the peak parameters presented above, this analysis strongly indicates that dynamin is involved in increasing the pore size, stability, and eventual dilation of the pore.

2.4. Inactivation of dynamin GTPase properties decreases the stability of the fusion pore

In some peaks, a pre-spike feature, or foot, was observed. The foot is generated by the formation of the fusion pore, and is a critical indicator of the pore dynamics.^{[19],[26],[28],[39]} Feet showing current i_{foot} above 1 pA were used for our analysis, and processed according to the procedure described in the Experimental section. The results obtained for this dataset are shown on Table 2.

Fewer feet were recorded in presence of 1 μM dynasore, indicating that this treatment makes the pore less stable, and increases its rate of dilation. This observation is in good agreement with the fact that dynasore decreases the foot time t_{foot} , leading to a smaller amount of molecules released N_{foot} . Interestingly, the foot current, i_{foot} , was not changed by the inhibition of dynamin activity. As this parameter is related to the pore diameter,^{[21],[4]} this suggests a short-lived unstable pore geometry, unchanged by the exposure to dynamin. This foot analysis suggests that the inhibition of dynamin GTPase action mostly alters the dynamics of the pore, rather than its initial geometry, and induces an unstable fusion pore, prone to dilate and close more quickly than in the control case.

2.5. Proposed role for dynamin during exocytosis

From the results presented above, inhibition of the GTPase activity of dynamin leads to the observation of smaller, shorter exocytotic peaks. This observation is in good agreement with results published recently from near field microscopy observation of pore dynamics, where the GTPase activity of dynamin was inhibited by dynasore or mutations.^{[9],[10]} The duration of the feet is also reduced, and the constraining effect of the pore is larger on the decaying part of the peak. In terms of pore dynamics, this indicates that the fusion pore opens and closes faster, and that its maximum opening radius is decreased.

Dynamin, in the presence of lipid nanotubes, spontaneously forms collar-shaped structures.^[6] The formation of the dynamin structure should not be hindered by the dynasore treatment, as this compound is targeted at the GTPase activity of dynamin.^[24] Therefore, the

dynamins should be present in the control and dynasore treated cells. The presence of GTP triggers the constriction of the dynamin constructs.^[8] This activity is critical for the late steps of endocytosis.^[24] In the presence of dynasore, endocytosis appears to be blocked at intermediate steps, as the neck of the vesicle cannot be broken to release the vesicle from the membrane.^[24]

The proposed role of dynamin is summarized on Figure 6. In the case of extended kiss and run exocytosis, it appears that the dynamin complex actually contributes to supporting, or framing, the nanotube formed between the vesicle and the membrane. In this case, the pore does not follow its intrinsic kinetics, but would actually be controlled by the dynamin complex. The longer t_{foot} in presence of the fully active dynamin possibly indicates that the foot feature corresponds to the formation of the dynamin assembly and the initiation of the GTPase activity, followed by the pore expansion. Our results, in good agreement with others,^{[9],[10]} suggest that the mechanochemical activity of dynamin is involved in exocytosis, and that this activity relies on its GTPase properties.

It has recently been suggested that the actin cytoskeleton actually promotes the closing of the pore.^{[40],[41]} In this case, a system constituted uniquely of the SNARE proteins, the cell membrane and the actin cytoskeleton would mostly lead to hastily closing fusion pores. The mechanochemical activity of the active dynamin complex would, in this case, help to counteract pressure induced by the cytoskeleton on the pore and lead to larger peaks than in the absence of the GTPase activity of dynamin. The inhibition of the exocytotic activity in presence of high concentrations of dynasore actually indicates that the effect of the cytoskeleton is probably potent enough to completely block the formation of the pore, and the exocytotic release.

3. Conclusions

The GTPase properties of dynamin have a direct effect on the duration and kinetics of exocytotic release. The inhibition of this specific feature of dynamin lead to shorter, smaller amperometric peaks for exocytosis. Interestingly, the role of dynamin in exocytosis appears to be the opposite of its role in endocytosis, as its mechanochemical properties appear, at least in part, to contribute to the dilation and stability of the pore. This observation strongly hints that dynamin protects the pore from other closing forces applied to the lipid membrane. The actin cytoskeleton, the SNARE complex, and the lipid composition of the membrane might be major factors interacting with dynamin and controlling the rate of the exocytotic efflux.

Experimental Section

Chemicals and solutions

The chemicals, of analytical grade, were obtained from Sigma-Aldrich (unless stated otherwise) and used as received.

The HEPES physiological saline contains 150 mM NaCl, 5 mM KCl, 1.2 mM MgCl₂, 5 mM glucose, 10 mM HEPES, and 2 mM CaCl₂. The K⁺ stimulating solution consists of 55 mM NaCl, 100 mM KCl, 1.2 mM MgCl₂, 5 mM glucose, 10 mM HEPES, and 2 mM CaCl₂.

All solutions were made using 18 M .cm water from a Millipore purification system and the solutions pH was adjusted to 7.4 with concentrated (3 M) NaOH.

Fabrication of the disk microelectrodes

The fabrication of these electrodes was previously described.^[25] Briefly, the carbon fiber working electrodes were fabricated by aspirating 5 μm diameter carbon fibers into borosilicate glass capillaries (1.2 mm O.D., 0.69 mm I.D., Sutter Instrument Co., Novato, CA). The capillaries were subsequently pulled with a commercial micropipette puller (Model PE-21, Narishige, Inc., London, UK) and sealed with epoxy (Epoxy Technology, Billerica, MA). After beveling (Model BV-10, Sutter Instrument Co., Novato, CA) at 45 °, each electrode was then tested by performing cyclic voltammograms in a solution of 0.1 mM dopamine in PBS (pH 7.4). Only the electrodes showing good reaction kinetics and a diffusion limited current in agreement with the theoretical value calculated for a 5 μm diameter disk were used for the experiments.

Cell culture

PC12 cells were purchased from the American Type Culture Collection (Manassas, VA). The cells were maintained in phenol red-free RPMI-1640 media (PAA Laboratories, Inc. Australia) supplemented with 10 % donor equine serum (PAA Laboratories), 5 % fetal bovine serum Gold (PAA Laboratories), 2 mM L-glutamine and 0.4 % penicillin streptomycin solution (PAA Laboratories) in a 7 % CO₂, 100 % humidity atmosphere at 37 °C. The cells were grown on mouse collagen coated cell culture flasks (collagen type IV, BD Biosciences, Bedford, MA) and were sub-cultured every 7-9 days. The medium was replaced every 2 days throughout the lifetime of all cultures. For stimulated exocytosis experiments at single cells, PC12 cells were grown on mouse collagen coated culture dishes (type IV, BD Biosciences, Bedford, MA) 4-5 days before experiment and cell media was replaced every day.

Single cell experiments

Electrochemical recordings of exocytotic events from single PC12 cells were performed as previously described^[26] on an inverted microscope (IX71, Olympus), in a Faraday cage. The working electrode was held at +700 mV versus an Ag|AgCl reference electrode (Scanbur, Sweden) using an Axon 200B potentiostat (Molecular Devices, Sunnyvale, CA). The output was filtered at 2.1 kHz using a Bessel filter and digitized at 5 kHz. Before experiments, the cells were rinsed three times with HEPES buffer at 37°C and were maintained under these conditions throughout the experiment. A glass micropipette containing K⁺ stimulating solution was positioned 60 μm from the cell. Each cell was then stimulated once with a single 5 s K⁺ injection (20 psi) through the micropipette coupled to a microinjection system (Picospritzer II, General Valve Corporation, Fairfield, NJ). A constant potential (700 mV) was applied to the microelectrode with respect to the Ag|AgCl reference electrode placed in the cell bathing solution throughout the experiment. All cell experiments were performed at 37 °C.

The cells were exposed to dynasore by rinsing the dishes 3 times with HEPES saline and incubating the cells for 5-10 min, in the humid incubator, with a solution of dynasore in HEPES buffer.^[27] The 100 mM stock solution of dynasore was prepared in DMSO, and the appropriate amount of this solution was dissolved in warm HEPES. Different final concentrations of dynasore, ranging from 100 nM to 100 μM were tested. After the 5 min incubation, the cells were immediately tested, and the dynasore was left in the supernatant during the electrochemical experiments.

Data processing and statistics

All the data processing routines were performed with IgorPro 6.21. The amperometric traces were processed using an IgorPro 6.21 routine originating from David Sulzer's group.^[28] The

filters for the current and differentiated current traces were 2 and 1 kHz, respectively. The threshold for peak detection on the differentiated trace was three times the standard deviation of the noise. The traces were carefully inspected after peak detection and false positive were manually rejected. The fitting of the peak parameters was adjusted. All the peaks larger than 2 pA (about four times the noise of the smoothed signal, between 0.5 and 0.7 pA in our experiments, based on 2- to 4-s baseline acquisitions at the beginning of the trace) were collected. Feet showing a foot current higher than 2 pA were selected for analysis.

The parameters obtained from the peaks are, for the body of the peak, the peak current, i_p , the rise time, t_{rise} , defined as the time separating 25 % of the maximum from 75 % of the maximum on the ascending part of the spike, the half peak width, $t_{1/2}$, defined as the width of the exocytotic peak at half of its magnitude, the fall time, t_{fall} , defined as the time separating 75 % of the maximum from 25 % of the maximum on the descending part of the spike, and the charge, N , *i.e.* the area under the curve, expressed as a number of molecules (see Figure 2). For the feet, the parameters are the foot current, i_{foot} , defined as the average of the measured current over the duration of the foot or the plateau current when a steady-state is reached, the foot duration, t_{foot} , and the foot charge, N_{foot} , *i.e.* the area under the curve defining the foot, expressed as a number of molecules.

These parameters were pooled, and the median of the data was calculated. The distribution of the sample was evaluated using the 1st quartile–3rd quartile (or 25 %-75 %) interval. The distribution of the exocytotic parameters is asymmetric and strongly deviates from normality, hence motivating the use of the median in place of the mean.^{[14],[17],[28],[29]} Additionally, the use of the median was found to minimize the impact of the cell-to-cell variations, as the value is less sensitive to outliers. Pairs of datasets were compared with the two-tailed Wilcoxon-Mann-Whitney rank-sum test, ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$.^[2] The threshold for significance for the peak parameters was $p < 0.01$, and $p < 0.05$ for the foot parameters. A lower threshold value had to be set for the peak analysis, because of the low sample number (only ~10% of the peaks featured a foot).

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Figure 1.

Full distension *vs.* extended kiss and run. In the case of a full distension event, the vesicle is completely integrated into the membrane, and all its content is released in the process. During extended kiss-and-run, the fusion pore closes again after partially expanding, possibly interrupting the release of neurotransmitters before the end of the event. It is expected that the vesicle is reloaded and used again.

Figure 2.

Dynasore inhibits exocytosis. The fraction of cells generating at least one exocytotic peak over the number of cells tested is plotted as a function of the dynasore concentration (logarithmic scale). The curve was fitted with a sigmoid. In the control case where no dynasore is added, 77% of the cells were active.

Figure 3. Analysis of the exocytotic peaks. Definition of the peak current, i_p , the rise time, t_{rise} , the half peak width, $t_{1/2}$, the fall time, t_{fall} , and the charge, N , *i.e.* the area under the curve, used to characterize the peaks; on the left, the foot parameters t_{foot} and i_{foot} are detailed.

Figure 4.

Average peaks obtained from typical for the control and 1 μ M dynasore treatments. The peak parameters obtained for these treatments are detailed on Table 1.

Figure 5.

Dynasore treatment increases the prevalence of double exponential decays. A) Scatter plots showing T_1 vs. T_2 as defined by equation 1, showing a $T_1 = T_2$ sub-population indicated by a line for $T_1 = T_2$; B) scheme detailing the calculation of the normalized distance d between the point (T_1, T_2) and the line describing the identity function; C) plots of d vs. the fraction of population n/N_{tot} for the control and 1 μ M dynasore treatments.

Figure 6.

Proposed role of dynamin during exocytosis in the case of extended kiss and run exocytosis. Top) When dynamin is active, the formation of the pore triggers the cross-linking of dynamin into a coil supporting the lipid nanotube. This coil frames the pore, leading to a wider pore radius and a higher i_p . Additionally, the pore is open for a longer time. Bottom) If dynamin is blocked, the lipid nanotube is now controlled by its own intrinsic dynamics. As the pore is not framed by the dynamin coil, it does not open as widely as in the control case, and tends to collapse more rapidly.

Table 1

Experimental results for $t_{1/2}$, t_{rise} , t_{fall} , i_p , and N obtained from K^+ -stimulated PC12 cells, comparing control (23 cells, 702 peaks) to dynasore-treated cells (100 nM: 13 cells, 238 peaks; 1 μ M: 16 cells, 94 peaks).^a

Treatment	t_{rise}/ms	$t_{1/2}/ms$	t_{fall}/ms	I_p/pA	$N/10^3$ molecules
Control	0.9 (0.5-2.1)	3.7 (2.8-5.2)	3.4 (2.3-5.3)	5.1 (3.6-7.4)	88 (61-148)
100 nM dynasore	0.8 (0.5-1.7)	2.7 (1.9-4.0)	2.5 (1.6-4.1)	4.9 (3.1-10.2)	74 (52-118)
Variations	-13%	-27% ***	-28% ***	-4%	-17% ***
1 μ M Dynasore	0.8 (0.4-2.1)	2.1 (1.1-3.4)	2.1 (0.9-4.2)	4.2 (3.3-5.3)	63 (32-120)
Variations	-12%	-43% ***	-38% ***	-17% ***	-28% ***

^aThe data are presented as median (1st quartile-3rd quartile). The pairs of datasets were compared using a two-tailed Wilcoxon-Mann-Whitney rank-sum test,

: $p < 0.001$;

**
: $p < 0.01$.

The variations of the median in comparison to the control are also reported.

Table 2

Foot parameters obtained from K⁺ stimulated PC12 cells. The data is presented as median (1st quartile-3rd quartile). ^a

Treatment	i_{foot} pA	t_{foot} ms	N_{foot} 10 ³ molecules	Peaks with a foot
Control	1.4 (1.2-2.0)	3.9 (1.8-7.8)	21 (11-41)	12%
100 nM dynasore	1.3 (1.2-2.0)	2.6 (1.3-4.3)	14 (6-25)	13%
Variations	-2%	-35% *	-34% *	
1 μ M Dynasore	1.5 (1.4-1.6)	1.5 (1.0-2.5)	9 (6-12)	7%
Variations	+9%	-62% *	-58% *	

^aThe pairs of datasets were compared using a two-tailed Wilcoxon-Mann-Whitney rank-sum test,

* : p < 0.05.

The variations of the median in comparison to the control are also reported.