‘Full fusion’ is not ineluctable during vesicular exocytosis of neurotransmitters by endocrine cells

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1. Introduction

The significance of vesicular exocytosis in biology and medicine is evidenced, among many other examples [1–6], by two recent Nobel Prizes in Physiology and Medicine, the latest one being awarded in 2013 to James E. Rothman, Randy W. Schekman and Thomas C. Südhof [3–6]. In neurons and endocrine cells neurotransmitters are transported as cargo by vesicles, tailored and loaded in the Golgi apparatus and delivered at specific release sites of the cell membrane where they dock to finally release neurotransmitters through a fusion pore connecting the cell and vesicle membranes [7–12].
In endocrine cells, release through the initial fusion pore is minute and the pore may close or flicker (Kiss and Run) [13–21], as occurs in neurons [22–24]; however, generally the pore rapidly expands [25] to release a massive flux of neurotransmitters that is precisely quantifiable by amperometry at carbon-fibre micro- and nanoelectrodes [22–24,26–34]. This stage is generally considered to end in the full integration of the vesicle membrane into the cell membrane, hence its ‘full fusion’ designation. There is a wealth of data characterizing in detail vesicle formation and transport, SNARE-assisted docking stages as well as the initial fusion pore size and flickering dynamics. However, the ineluctability of the ‘full fusion’ stage has recently become a matter of debate [30–33], though, at least in endocrine cells, it conditions the ultimate purpose of the whole process leading to the release of neurotransmitters.

If the fusion pore dynamics is exclusively governed by bilipid membrane energetics [34–36], its enlargement should be driven by the viscous dissipation of tension energies imposed by the important curvatures created at the small fusion pore edges and by the vesicle membrane surface tension [32,34–36]. The replacement of catecholamine cations by hydrated monovalent ones inside the matrix during release should lead to swelling of the pore [37–40]. However, swelling is necessarily restrained by the presence of the vesicle membrane, at least while the pore radius remains small compared with that of the vesicle [32]. The ensuing internal swelling pressure that builds up in the constrained matrix during release provokes a constant increase in the membrane surface tension and sustains the continuous enlargement of the fusion pore [35,36]. So, in the absence of external factors, ‘full fusion’ appears ineluctable. This explains why the occurrence of this stage has become a paradigm in the field. In addition, several total internal reflection fluorescence microscopy (TIRFM) [25] and electron microscopy (EM) [15,41–43] data support its existence through reports of fusion pores with sizes comparable to those of vesicles.

However, it is not clear whether or not these data represent normal or exceptional events possibly related to another vesicle function [44–47]. Actually, in endocrine cells, depending on the level of excitation and the size of the fusion pore [44–47], exocytotic vesicles ensure a second important function besides the release of small neurotransmitter molecules that is considered here. This second role serves to regulate hormone peptides through the release of the chromogranins forming the matrix structure [49–52], followed by their enzymatic digestion. Release of such long highly folded peptidic strands requires that the matrix is swollen and almost fully exposed to the extracellular fluid. This implies the formation of a fusion pore with size comparable to that of the former vesicle [44–47], as described by the ‘full fusion’ paradigm and observed by TIRFM and EM. This duality of function is fully coherent with the significant delays observed between fusion pore opening and peptide release by neuroendocrine cells’ dense-core vesicles [44–47,52]. Conversely, neurotransmitters are small enough to diffuse within still compact matrices and for high fluxes to be released through much smaller fusion pores.

Amperometry at carbon-fibre micro- and nanoelectrodes (artificial synapse) [26,27] allows statistically relevant series of kinetic measurements of neurotransmitter fluxes emitted by endocrine cells [26–33] or in neuronal synapses [22–24] to be recorded as soon as the initial fusion pore opens. Notwithstanding the considerable wealth of information provided by amperometry [27,30], relating fluxes to fusion pore sizes has been unmanageable until recently. Doing so requires knowledge of the transport rate of the neurotransmitters within vesicle matrixes, \(\kappa = \frac{D_{\text{ves}}}{R_{\text{ves}}^2}\), where \(D_{\text{ves}}\) is the apparent diffusion coefficient of the neurotransmitter inside the vesicle matrix of radius \(R_{\text{ves}}\) [53–55]. Yet, \(\kappa = 415\ \text{s}^{-1}\) was determined recently for chromaffin

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1It must be noted that, due to spatial resolution of the two methods, fusion pores must have expanded to a sufficiently large size to be observable by TIRFM or EM. Hence, fusion pores with radii much smaller than those of vesicles may then appear in TIRFM or EM as featuring vesicles in a pre-release docking stage [25] or as undergoing Kiss and Run. Hence, small fusion pores may be common but undetectable by these methods, even though they give rise to massive neurotransmitter release that is detectable by amperometry. In this respect, it is noted that coupled TIRFM–amperometry experiments evidenced poor time correlations between events expected to be characterized simultaneously by the two methods (e.g. [48]). Even so, conclusions based on amperometry, TIRFM and EM are not necessarily contradictory. Indeed, whether a fusion pore closes or enlarges after neurotransmitter release ends cannot be inferred from most amperometric measurements whenever the current has reached the baseline level before this occurs.

2Transport inside a still compact matrix may not proceed through classical diffusion (i.e. in the Einstein–Schmoluchowski sense) even across tortuous pathways, but through site-hopping between occupied and free sites. However, following the
cells [55] based on correlations between current pre-spike-feature intensities and the initial fusion pore size, $R_{\text{initial}}$, values reported by patch clamp [20]. This allowed quantitative extraction of the time course and final values of fusion pore expansion from individual amperometric spike currents based on heavy and delicate auto-adaptive simulations [23,53–55]. Although manageable with difficulty by non-experts due to the requirement for human decision at several critical stages [23,54,55], such simulations established quantitatively for the first time that most amperometrically detected events involve fusion pores whose expansion was stalled at a maximum size of only approximately one-tenth of that of the vesicle. These results, as well as other evidence inferred by others based on purely experimental strategies [30,31,66–69], have instilled the concept that, in endocrine cells, most releasing events proceed entirely through fusion pores whose radii remain much smaller than those of vesicles, i.e. they contradict the ‘full fusion’ paradigm.

These previous simulations provided important knowledge about the dynamics of diffusion within endocrine matrixes during catecholamine release (see a summary of the main points and conclusions in the electronic supplementary material, S1 and S2). One key conclusion is that most of the release is governed by a quasi-steady-state diffusional regime that is achieved within the vesicle from the very beginning of release. This conclusion leads to a fast, simple and expedient analytical approach for extracting the time variations of the fusion pore radius, $R_{\text{pore}}(t)$, that we wish to demonstrate here. This method allows statistically significant sets of data to be gathered from control cells or from cells submitted to different strains that will then be used to examine whether or not fusion pore expansion is solely regulated by the energetics and dissipative properties of the cell–vesicle membrane assembly. For this purpose, we will take advantage of several sets of amperometric spikes that were published previously by our group [70,71] and whose classical amperometric characteristics are summarized in the electronic supplementary material, S3.

2. Results and discussion

It is now well established that almost all amperometric spikes involve exponentially decaying branches [53–55].\(^3\)\(^4\) The origin of such behaviour is a direct consequence of the quasi-steady-state diffusional regime established within the vesicle (see electronic supplementary material, S2) when the fusion pore has reached its maximal opening size (see below). In this work, we need to restrict ourselves to the commonly considered situation in which the current decays following a single-exponential mode, although it is noted that a non-negligible fraction of events involve decay branches exhibiting two-term exponential modes [30,32,33,66,69]. Although the origin of this second class of spikes has been fully rationalized [32], the method developed hereafter cannot be directly applied to such events.

Our previous works [53–55], whose main conclusions are summarized in the electronic supplementary material, S1 and S2, established that when $\kappa t > 0.1$ (that is, $t > 0.1 \times R_{\text{ves}}^2/D_{\text{ves}} \approx 0.2 \text{ms for } \kappa = 415 \text{ s}^{-1}$ [55]), i.e. under all experimental circumstances of interest here, the diffusively controlled neurotransmitter concentration pattern established within a releasing vesicle reaches a quasi-steady-state regime. Under this regime, at any time the concentration at any point within the matrix is proportional to its time-dependent neurotransmitter average value within the whole matrix, with a scaling factor that depends on the location within the matrix but is independent of time (see electronic supplementary material, figure S1). Note that this property of diffusional leakage from a closed reservoir is universal and is the justification for the Newton

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\(^3\)The first experimental report of the systematic occurrence of exponential tails for amperometric spikes was provided by Brioso et al. [72].

\(^4\)It is noted that this is also fully consistent with the long time limit of the empirical equation (5) reported in [73], which was used in the earliest semi-empirical quantitative analyses of amperometric spikes recorded during stimulated release in chromaffin cells.
and Kelvin exponential laws of cooling of solid bodies. It then follows that at any time, \( t \), the flux of catecholamine cations through the fusion pore (i.e. the concentration gradient at the entrance of the pore) is proportional to the average quantity, \( q_{\text{ves}}(t) \), of releasable catecholamines still present inside the vesicle matrix at the same time, \( t \). Hence, assuming that the released fluxes are exclusively governed by the convergent diffusion of neurotransmitters inside the matrix towards the entrance of the fusion pore (see the electronic supplementary material, S1, for justification of this model based on our previous works [32,33,53–55]) allows us to express the time-dependent quantity of releasable catecholamines inside the matrix as follows:

\[
\frac{dq_{\text{ves}}}{dt} = -\kappa \rho \times q_{\text{ves}}(t),
\]  

where \( \rho \) is a time-dependent coefficient that depends on the value of \( R_{\text{pore}}(t)/R_{\text{ves}} \). This justifies the observation of amperometric spikes with single-exponential decay current branches. Indeed, as soon as the fusion has reached its time-independent final size \( R_{\text{pore}}^{\text{max}} \), the product \( \kappa \rho \) becomes a constant. Accordingly, \( q_{\text{ves}} \) decreases exponentially, giving an indirect proof of the quasi-steady-state diffusional regime. On the other hand, owing to Faraday’s law, the amperometric current is given by

\[
i(t) = -2F \left( \frac{dq_{\text{ves}}}{dt} \right),
\]

as the oxidation of any catecholamine molecule at the electrode surface consumes two electrons [27]. Hence, the amperometric spike currents also decay exponentially.

Interestingly, \( \rho \cong R_{\text{pore}}(t)/R_{\text{ves}} \) provided that \( R_{\text{pore}}(t)/R_{\text{ves}} < 0.7 \) [32,33,53–55], a condition that applies to all amperometric events treated hereafter (see below), so that equation (2.1) is written as

\[
\frac{dq_{\text{ves}}}{dt} = -\kappa \left[ \frac{R_{\text{pore}}(t)}{R_{\text{ves}}} \right] dt.
\]

Noting \( q_{\text{ves}}^{\text{tot}} \) as the total amount of releasable catecholamine cations in the vesicles, namely

\[
q_{\text{ves}}^{\text{tot}} = \frac{1}{2F} \int_{0}^{\infty} i(t)dt,
\]

as follows from equation (2.2), equation (2.1) can be rewritten as

\[
R_{\text{pore}}(t) = \frac{[i(t)/\kappa]}{[2Fq_{\text{ves}}^{\text{tot}} - \int_{0}^{\infty} i(u)du]},
\]

that is,

\[
R_{\text{pore}}(t) = \left( \frac{R_{\text{ves}}}{\kappa} \right) \times \left[ \frac{i(t)}{\int_{0}^{\infty} i(u)du} \right]
\]

(note that in equations (2.5) and (2.6) the variable \( u \) represents a dummy integration variable related to the time).

By definition, equation (2.6) applies as soon as \( t > 0.1/\kappa \), i.e. as soon as the quasi-steady-state regime is achieved within the vesicle under scrutiny. Its validity was tested by comparison with the results of our previous rigorous auto-adaptive numerical procedures [23,53–55] and was found to be accurate within 1% at worst. For \( \kappa = 415 \ \text{s}^{-1} \) as determined previously, equation (2.6) applies at times larger than approximately 0.2 ms after the beginning of release, that is, it virtually describes the whole spikes and their possible pre-spike features (PSFs) that correspond to the opening of the initial fusion pore and are observed in approximately 30% of the events [27,30].

For determining \( \kappa \) in [55], we relied on (i) the mean current intensities of the pre-spike features and (ii) the \( R_{\text{pore}}^{\text{initial}} = 1.2 \ \text{nm} \) value measured by patch clamp for the radius of the initial fusion pore [20]. If the patch-clamp community re-evaluates this value by a factor \( \gamma \), all \( R_{\text{pore}}(t) \) values reported in this work need to be rescaled by the same factor \( \gamma \) as \( \kappa \) is, by construction of the extraction procedure [55], proportional to \( 1/R_{\text{pore}}^{\text{initial}} \) while \( R_{\text{pore}}(t) \) is proportional to \( 1/\kappa \); see equation (2.6). Hence, the absolute values reported here are ultimately proportional to the size adopted for \( R_{\text{pore}}^{\text{initial}} \). Interestingly, using \( R_{\text{ves}} = 156 \ \text{nm} \) for the mean vesicle radius [74], \( \kappa = 415 \ \text{s}^{-1} \) corresponds to \( D_{\text{ves}} = 10^{-7} \ \text{cm}^{2} \ \text{s}^{-1} \), i.e. to a value approximately 50 times less than in an aqueous medium, a fact that seems consistent with a polyelectrolyte gel. Note that, for each individual event, \( \kappa \) is susceptible to variations with an exponential distribution [55]. This is the reason for our reliance on the statistical analyses of hundreds of spikes, for which these random variations should compensate. In other words, the \( R_{\text{pore}}(t) \) values reported in figure 1a(i–v) would be better viewed as illustrative as each may suffer a small random scaling factor. Conversely, the data reported in figure 1b(i–v),c(i–v) should be immune to such random effects through statistical auto-compensation.
of the PSF that represents, when observable, the opening of the initial fusion pore. Indeed, then the release kinetics are certainly governed in part by the transport through the sub-nanometric channel and not only by the convergent diffusion inside the matrix. Indeed, the only hypothesis made in deriving equation (2.6) amounts to assuming that the rate of transit across the fusion pore channel is not rate limiting, i.e. that the released flux only depends on the concentration gradients inside the vesicle at the entrance of the fusion pore [54,55,75–77]. As recalled in the electronic supplementary material, S1, this is valid as soon as the initial fusion pore has achieved its nanometric initial radius [20], but probably not before this is achieved.

Finally, it is remarked that an independent knowledge of the $R_{ves}/\kappa$ value is not required for applying equation (2.6) to a series of related individual current spikes that involve releasing events from essentially similar vesicles, that is, belonging to a given cell type. Indeed, if this factor is unknown, $R_{pore}(t)$ could be obtained on the same relative scale. In the following, $R_{pore}(t)$ variations are reported on an absolute scale through relying on the mean $R_{ves}=156\text{nm}$ vesicle radius reported for chromaffin cells [74] and $\kappa=415\text{s}^{-1}$ as determined previously [55] (see footnote 5). Indeed, the size of the initial fusion pore that led to the measurement of $\kappa=415\text{s}^{-1}$ for chromaffin cells is imposed by the architecture of the SNARE assembly. Hence, this can be considered invariant even when the membrane is affected by brief external changes in experimental conditions (e.g. osmolality or trans-insertion of exogenous lipids; see below).

Two quantitative features are useful for characterizing these curves. One is the maximal size, $R_{pore}^{\text{max}}$, of the fusion pore (figure 1a,b) and the other its radial expansion rate, $v_{\text{open}} = dR_{pore}(t)/dt$, represented by its maximum value, $v_{\text{open}}^{\text{max}}$, in figure 1c. Within the ‘full fusion’ paradigm one would expect that $R_{pore}^{\text{max}}/R_{ves} \to 1$ while a significant amperometric current is still monitored. Figure 1a,b establishes that this is far from being the case: under all circumstances examined here, $R_{pore}^{\text{max}}$ median values are approximately one-tenth of the mean vesicle radius and always
remain less than approximately one-fifth of it. This same upper limit was observed for controls (figure 1b(i)) or for cells submitted to brief hypertonic (figure 1b(ii)) or hypotonic (figure 1b(iii)) shocks [70], although the membrane surface tension was drastically reduced in the first case and drastically increased in the second one compared with controls (see the electronic supplementary material, figure S2a–c). These perturbations affect the shapes of $R_{\text{pore}}^{\text{max}}$ distributions in figure 1b (see table 1 for the median and quartile values), increasing the probability of small $R_{\text{pore}}^{\text{max}}$ values under hypertonic conditions (figure 1b(ii), membranes with low tension) and that of large ones for hypotonic conditions (figure 1b(iii), membranes with high tension). From a mechanical perspective, both changes are in agreement with the expected decrease or increase, respectively, of the surface tension, i.e. of the driving force powering the fusion pore expansion [34–36, 75–77]. Nonetheless, one observes that $R_{\text{pore}}^{\text{max}} < 30 \text{ nm}$ in all cases (normal, hypertonic or hypotonic conditions), suggesting that this limit does not depend on the membrane characteristics, although, as expected, it is more frequently reached for tense membranes than for relaxed ones.

Another set of experiments involved cells submitted to brief micromolar incubations with exogenous bilipids with cone angles $\theta$ different from that, $\theta \approx 0$, of the ‘cylindrical’ endogenous cell ones, namely, arachidonic acid (AA) (‘cone-shaped’, $\theta_{\text{AA}} > 0$; figure 1b(iv)) or lyso-phosphatidylcholine (LPC) (‘inverted cone shaped’, $\theta_{\text{LPC}} < 0$; figure 1b(v)). These brief incubations were performed immediately before stimulating release [71], with the intention of altering the fusogenic and dissipative properties of the membrane assembly [78–83]. The brief incubations ensured that exogenous lipids exclusively trans-inserted in the outer leaflet of the cell membrane [82], with opposite consequences for the large positive curvatures at the fusion pore edges [36,53–55,75–77,84,85] due to their shapes. Figure 1b(iv) confirms the unfavourable effect of the negative curvatures promoted by AA [84,85]. By contrast, as expected, LPC favours positive curvatures [82,83] and shifts $R_{\text{pore}}^{\text{max}}$ distributions towards larger values (figure 1b(v) and table 1). Nonetheless, in both cases, the previous limit at approximately 30 nm holds. This ubiquitous limit strongly suggests that, while it expands, the fusion pore tube external wall encounters a biological barrier that blocks its further enlargement beyond this range [86,87]. Considering that, in this work, we access the inner radius of the fusion pore, taking into account that the thickness of a lipidic bilayer ranges between 3 and 4 nm, and neglecting the possible presence of membrane proteins in the fusion tube, the probable diameter of the free space in which the fusion pore may expand is approximately 50–70 nm under control conditions. This is large but remains between approximately one-sixth and one-quarter of the mean vesicle diameter (312 nm [74]). Still, it is noted that such a value is not incompatible with the mesh sizes of sub-membrane cytoskeleton proteic structures [88–91].

<table>
<thead>
<tr>
<th>conditions$^c$</th>
<th>$R_{\text{pore}}^{\text{max}}$ (nm)$^b$</th>
<th>$v_{\text{open}}^{\text{max}}$ ($\mu \text{m s}^{-1}$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>controls</td>
<td>16.8 (12.6; 20.2)</td>
<td>1.41 (0.74; 2.41)</td>
</tr>
<tr>
<td>hypertonic</td>
<td>10.6 (6.6; 15.9)</td>
<td>0.74 (0.35; 1.84)</td>
</tr>
<tr>
<td>hypotonic</td>
<td>20.3 (16.8; 21.9)</td>
<td>1.42 (0.93; 2.21)</td>
</tr>
<tr>
<td>AA-modified</td>
<td>11.4 (8.2; 15.3)</td>
<td>0.66 (0.40; 1.14)</td>
</tr>
<tr>
<td>LPC-modified</td>
<td>17.0 (15.0; 20.1)</td>
<td>1.65 (1.32; 2.42)</td>
</tr>
</tbody>
</table>

$^a$Vesicular release elicited from chromaffin cells by injection of 2 mM Ba$^{2+}$ (in Locke buffer supplemented with 0.7 mM MgCl$_2$ without carbonates) during 2 s.

$^b$Median values (first and third quartile values reported between parentheses) based on equation (2.6) with $R_{\text{ves}} = 156$ nm [74], and $\kappa = D_{\text{ves}}/R_{\text{ves}}^2 = 415$ s$^{-1}$ [55] (see text).

$^c$Amperometric spikes measured at bovine chromaffin cells with 7 $\mu$m-diameter carbon-fibre microelectrodes held at 0.65 V versus Ag/AgCl; see electronic supplementary material, S3 and [70,71] for experimental details.
Before the fusion pore external edge meets this limit, its radial expansion rate results from a balance between the driving force acting on the edge of the pore and the ability of the system to relax its released energy through viscous dissipation \([35,36,75,76]\). Accordingly, \(R_{\text{pore}}(t)\) enlarges exponentially at the beginning of its expansion (i.e. before the markers in figure 1a) \([35,36,75,76,88–91]\). After this phase, the rate of expansion progressively levels off while the fusion pore radius reaches its maximum value, \(R_{\text{pore}}^{\text{max}}\) (figure 1c) provides a good indication of the expansion velocity before the fusion pore edge can start to significantly interact with the non-lipidic biological structure(s) that ultimately limit(s) its expansion.\(^6\)

All \(v_{\text{open}}^{\text{max}}\) distributions display exponential tails at large values, as is expected for individual rates of single events controlled by a single elementary process \([93,94]\). For hypertonic and AA-modified conditions this applies over the whole range of \(v_{\text{open}}^{\text{max}}\) values (figure 1c(i),(c),(iv)). Conversely, for controls and hypotonic and LPC-modified conditions (respectively, in figure 1c(i), (iii) and (v)) the probability densities in the low \(v_{\text{open}}^{\text{max}}\) range are considerably smaller than expected through extrapolating the upper-range exponential behaviour. This phenomenon is exacerbated for hypertonic and LPC-modified conditions compared with controls. As yet, we do not have a definite explanation for the occurrence of such behaviour, but this and the relative effects of the hypertonic and LPC-modified conditions compared with controls suggest that at least two factors control the initial fusion pore expansion rate \([94]\). One possible rationale amounts to considering that the decrease in leaflet–leaflet viscosity \([35]\) due to a high initial surface tension at the end of the SNARE-constricted phase (controls and hypotonic conditions, figure 1c(i) and (iii)) or to a stabilization of positive curvatures (LPC-modified conditions, figure 1c(v)) facilitates the viscous dissipation of the energy accumulated in the matrix before the fusion pore can expand, and hence decreases the likelihood of low \(v_{\text{open}}^{\text{max}}\) values. Still, whatever the exact mechanism(s) underlying such peculiar \(v_{\text{open}}^{\text{max}}\) distributions, the data in figure 1c establish that, in all cases, the rate of fusion pore expansion during the first part of its total span strongly depends on the bilipidic membrane properties, confirming what was inferred from \(R_{\text{pore}}^{\text{max}}\) distributions (figure 1b and table 1). However, this never leads to ‘full fusion’, as would happen if the expansion was controlled only by the membrane properties. Indeed, the fast initial enlargement of the fusion pore is rapidly counteracted by other forces that eventually limit its final radius at approximately 30 nm.

These two series of paired experiments confirm that the membrane tension forces act together to power fusion pore enlargement \([34–36,75–77]\) after its initial SNARE-stabilized architecture breaks down \([13]\). However, the internal pressure within the matrix necessarily continues to build \([37–40]\) during the whole release, thus contributing to increasing the surface tension forces even after the fusion pore has reached its maximum value. Nonetheless, these forces are ultimately insufficient to overcome those imposed by the biological barrier(s) \([86,87]\). This conclusion is in perfect agreement with recent reports from Ewing and co-workers \([68,69]\), who showed that interfering with proteins that are generally considered to be involved in vesicle budding (e.g. actin-regulating proteins or other cytoplasmic ones such as dynamin) modifies the intensity and time duration of amperometric spikes. In some respects, this also agrees with the possible interplay between dynamin and myosin described by Smith and co-workers \([44–47]\) to account for the dichotomy between catecholamine and peptide release by endocrine cells. Although recent reviews have still presented such possibilities as ‘hypothetical’ \([86,87]\), the measurements reported in figure 1a,b provide for the first time strong quantitative grounds for these views.

3. Conclusion

Altogether, the data presented here provide the first quantitative support for a new paradigm and reject the ineluctability of a ‘full fusion’ outcome when the fusion pore enlarges beyond its SNARE-stabilized architecture. If the rapid expansion of the fusion pore is unquestionably

\(^6\)In all cases, \(v_{\text{open}}^{\text{max}}\) values are compatible with those recorded for pore dynamics in tensed giant unilamellar vesicle bilayer membranes \([36]\). It is also noted that \(v_{\text{open}}^{\text{max}}\) values are considerably smaller than sound velocity in bilipidic membranes (e.g. \([92]\)), confirming that the expansion rates are regulated by viscous dissipation of membrane tension energies \([36,77]\).
promoted by the viscous dissipation of the edge and surface tension energies of the vesicle–cell membrane assembly, the corresponding driving forces are rapidly counteracted by other forces that apply as soon as the fusion pore radius reaches approximately 15–30 nm [30,66–69]. Within this perspective, the occurrence of the much wider fusion pores observed by TIRFM or EM, a fact that usually substantiates the ‘full fusion’ paradigm, may be featuring either incidental rare events or exocytotic vesicle functions that are not related to neurotransmitter release but possibly to another role such as hormonal peptide regulation [44–47,49–52].

Data accessibility. The experimental data used in this work were previously published [70,71].

Authors’ contributions. C.A. designed the research; I.S. and A.O. performed the research; A.O. wrote the computer codes; and C.A. prepared the manuscript.

Competing interests. The authors declare no competing interest.

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